A Life Course Approach to Potentially Modifiable Risk Factors for Poor Semen Quality

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ABSTRACT

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Poor semen quality is an indicator of male infertility and is also associated with a variety of adverse health outcomes in men. It is therefore important from both clinical and public health perspectives to discover predictors of poor semen quality, especially those that are potentially modifiable. My dissertation research focuses on two of these potential risk factors: adiposity and stress. Unlike most studies to date, which have only considered the relationship between these exposures and semen quality cross-sectionally, my research takes a life course approach. I explore associations between birth weight, adiposity in both childhood and adulthood, and allostatic load—a construct representing the effect of cumulative stress on the body's regulatory systems—and three commonly-used semen outcome parameters: sperm concentration, percent progressive motility, and percent normal morphology. The logic that underlies this approach is that while sperm are constantly being produced from sperm stem cells in the testes, which would argue in favor of cross-sectional studies, the sperm stem cells themselves and the Sertoli and Leydig cells that stimulate and nurture that metamorphosis are laid down in the fetal period and undergo important developmental and proliferative phases during early childhood and puberty that affect their number and functional health in adulthood.

Using data from 193 participants in the Study of the Environment and Reproductive health follow-up to the Child Health and Development Studies birth cohort, I was able to calculate birth weight for gestational age percentile (bw/ga) and six age-appropriate adiposity measures (at 4 months, 12 months, and 4 years, and in participants' 20s, 30s, and at the time of semen collection), then test for their independent, critical period, and cumulative associations with the three semen outcomes as well as a combined outcome measure of subfertility based on World Health Organization reference levels. While bw/ga was positively associated with sperm concentration, subsequent childhood adiposity measures showed increasingly negative associations, and none of the adult measures were significantly associated with concentration. By contrast, only adult measures were associated with motility and morphology. This suggests that there may be critical periods in childhood when adiposity negatively affects sperm

concentration by interfering with the development and proliferation of Sertoli and Leydig cells. Accumulation of oxidative stress in the testes due to overweight/obesity may explain the negative relationships between adult adiposity and sperm motility and morphology.

To investigate allostatic load's relationship to semen quality, I conducted a pilot study at Columbia University's Center for Women's Reproductive Care that enrolled 61 men who were having their initial diagnostic semen analysis and blood draw on the same day. Blood samples were analyzed for 7 biomarkers associated with homeostatic regulation across several physiologic domains. I then created an allostatic load scale in which participants were assigned 1 point for being in the high-risk quartile for systolic blood pressure, diastolic blood pressure, body mass index, or any of the biomarkers. In regression analyses, allostatic load was not associated with either sperm concentration or morphology, but showed an unexpected positive association with motility. This association was entirely driven by the six participants who scored 0 on the allostatic load scale and who did not differ from the rest of the sample in any way that could plausibly be linked to reduced motility. I therefore concluded that this was a spurious finding. In further analysis of the allostatic load variable itself, I found that few of its individual components were correlated with the semen outcomes. This contrasts with other studies of allostatic load and adverse health outcomes, but these have generally been conducted in either elderly or stressed populations, neither of which described my cohort. Allostatic load may not be a reliable measure of stress in reproductive age populations and may not capture regulatory systems appropriate to reproductive health outcomes.

My dissertation highlights the value and challenges of conducting semen quality research from a life course perspective. Future studies should consider collecting longitudinal data on adiposity and stress, as well as repeated semen samples beginning in adolescence in order to further our understanding of the natural progression of semen quality across the reproductive life span and provide the opportunity to explore whether modifying these risk factors affects semen quality.

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INTRODUCTION

I have divers times examined the same matter from a healthy man…not from a sick man…nor spoiled by keeping…for a long time and not liquefied after the lapse of some time…but immediately after ejaculation before six beats of the pulse had intervened; and I have seen so great a number of living animalcules…in it, that sometimes more than a thousand were moving about in an amount of material the size of a grain of sand.(1)

Antoni van Leeuwenhoek's startling description of thousands of "animalcules" in a drop of human semen marked a watershed moment in the study of human reproduction. Contrary to the prevailing belief at the time, which was that the egg contained all of the necessary elements for new life, Leeuwenhoek hypothesized that sperm contributed essential biological material to and played an equal role in embryonic creation(2). More than 300 years later, while Leeuwenhoek's hypothesis has been proven correct, much remains to be learned about factors that influence sperm formation, semen quality, and successful fertilization. In my dissertation research, I have set out to fill three gaps in our understanding of this much larger puzzle: 1) to systematically review the literature on body mass index (BMI) and all three main indicators of semen quality: sperm concentration, motility, and morphology, 2) to investigate the relationship of birth weight and of adiposity across the life course to semen quality, and 3) to explore the association between cumulative stress and semen quality.

Semen quality is an important topic for public health research for two main reasons. First, as Leeuwenhoek surmised, sperm are required for the creation of new human (and nonhuman—he went on to observe semen in many other animals) life, and poor semen quality can impair a man's biological ability to produce offspring. Second, semen can potentially provide a window into men's health, as poor semen quality has been linked to various adverse health outcomes. It has been suggested that poor semen quality may be like a canary in a coal mine, as it is a biological indicator that may signal a man's vulnerability to future health problems(3).

Male infertility is a common and costly public health problem.

It is generally—although not universally(4, 5)--held that poor semen quality, assessed by conventional measures that include concentration, motility, and morphology, is an indicator of male subfertility(6), also known as male-factor infertility. Male-factor infertility plays a role in approximately 50

percent of couples who seek infertility treatment annually in the United States(7), thereby contributing to the increasing use of expensive assisted reproductive technologies (ART). In 2013 (the most recent data available), 1.6% of all infants born in the United States (66,691 live births) were conceived via ART (range: 0.2% in Puerto Rico to 4.8% in Massachusetts)(8).

While ART has made it possible for infertile heterosexual couples—as well as single women and same-sex couples—to have genetically-related offspring, those offspring are at increased risk for adverse health outcomes compared to naturally-conceived children. Whether these adverse outcomes are associated with the treatment itself or the underlying subfertility that necessitated it is still unresolved. For example, while a 2015 meta-analysis of data from 50 cohort studies including 161,370 ART and 2,280,241 spontaneously conceived singleton pregnancies found that ART infants are substantially more likely than their non-ART counterparts to be of low (<2500 g) or very low (<1500 g) birth weight and to be born preterm (<37 completed weeks of gestation) or very preterm (<32 weeks)(9), a recent study of 272,551 singleton sibling pairs that included 1,813 pairs discordant on conception (ART vs. natural) concluded that differences in birth weight and preterm birth between ART and naturally-conceived infants could be explained by underlying maternal subfertility(10). Intracytoplasmic sperm injection, an additional procedure specifically indicated in cases of male-factor infertility, is associated with additional increased risk of congenital malformations, especially of the male urogenital system(11); transmission of genetic abnormalities related to male infertility, such as cystic fibrosis and Klinefelter syndrome(12); imprinting disorders(13); and autism(14).

Low birth weight and preterm infants are at increased risk of adverse perinatal and child health outcomes, including developmental delays, with consequent costs to individuals, families, and society. In 2005, the Committee on Understanding Premature Birth and Assuring Healthy Outcomes of the Institute of Medicine estimated the societal economic burden of preterm births—including medical care, early intervention, special education, and lost productivity--to be $26.2 billion(15), with the 4.6% of preterm and 5.0% of very preterm births associated with ART(8) accounting for more than $1 billion. ART infants are far more likely to be multiples than naturally-conceived babies (41.1% vs. 3.5% (8)), exponentially increasing delivery expenses and exacerbating the risk of prematurity and low birth weight associated with ART. Studies that suggest that the increased risk of adverse perinatal outcomes associated with ART

may derive from the underlying subfertility that indicated treatment rather than the treatment itself(16, 17) only underscore the importance of identifying and mitigating factors that compromise fertility.

Poor semen quality may also indicate an underlying health problem.

In 2001, building on decades of observational evidence of an association between poor semen quality and testicular cancer(18-20), as well as coincident declines in semen quality(21) and increases in testicular germ cell cancer in industrialized Western countries(22-24), Skakkebaek et al. coined the term "testicular dysgenesis syndrome" (TDS) to describe a constellation of urogenital symptoms including not only poor semen quality and testicular cancer, but hypospadias and cryptorchidism, as well(25). They and others have suggested that these symptoms might share a common etiology stemming from an environmental exposure (e.g., an endocrine disrupter) or secular trend (e.g., the rising prevalence of obesity)(25-29), which may be associated with additional adverse health outcomes. Recent research indicates that poor semen quality may also be linked to other cancers(30, 31), cardiovascular disease(32, 33), diabetes (reviewed in (34)), and overall comorbidity, as measured by the Charleston Comorbidity Index(32, 35). Semen quality has been found to be inversely associated with overall mortality in fertility clinic samples across different time periods and geographical settings(32, 33, 36, 37). While results for individual semen parameters vary across studies and disease types, the overall pattern suggests that poor semen quality may be a bellwether of elevated disease risk in men. Identifying and understanding potentially modifiable predictors of poor semen quality would therefore be a worthy public health goal.

Adiposity and stress may be risk factors for poor semen quality.

Studies that report associations between BMI and semen quality frequently make headlines in the popular press, but three published systematic reviews and meta-analyses have not been uniform in their findings(38-40). In Chapter 1, I conduct a qualitative systematic literature review of 56 English-language studies published prior to August, 2016, that include a statistical test of BMI and at least one of the three main semen quality measures: sperm concentration, motility, or morphology. Because papers in this area are notably heterogeneous in terms of study population (general vs. fertility clinic vs. proven fertile), participant age (young vs. mixed), sample size, and distribution of BMI within the study sample, I consider

whether results differ according to each of these criteria. I also map the sites of the various studies in order to ascertain whether study results are clustered by geographic region.

The vast majority of studies that consider BMI and semen quality are cross-sectional, and many justify that approach by the fact that the process of spermatogenesis occurs in the 74 days prior to ejaculation(41). But there is reason to hypothesize that adiposity at earlier periods in the life course—as well as the intrauterine environment itself—may influence the proliferation and development of Sertoli and Leydig cells in the testes that are responsible for sperm production and maturation and thereby affect semen quality. In Chapter 2, I explore this hypothesis by linking longitudinal data from selected male offspring from the Child Health and Development Studies pregnancy cohort to cross-sectional data from the Study of the Environment and Reproduction follow-up that contains semen quality measures from samples collected in middle age. In addition to analyzing whether birth weight for gestational age or any of six adiposity measures spanning early childhood and adulthood has an independent effect on sperm concentration, motility, or morphology, I test two models central to life course epidemiology theory(42): critical period and accumulation of risk. In a secondary analysis, I also explore whether trajectories of adiposity within childhood and adulthood, as well as across the life span, influence semen quality.

Numerous studies have analyzed the relationship between current stress and semen quality (summarized in Chapter 3), and while their measures of stress (and anxiety) have differed, they have all been recent or current and based on either psychosocial constructs or life events. Taking a life course approach once again, I hypothesize that cumulative stress might also be worth considering as a predictor of semen quality. In Chapter 3, I investigate the association between allostatic load—a scale based on biological measures that theoretically reflects the wear-and-tear of cumulative stress on the body's major homeostatic systems(43)—and sperm concentration, motility, and morphology. In addition to using the full 10-item allostatic load scale, I also assess correlations between its individual components and semen quality in order to determine whether they work in sync, as Seeman et al. found in their study of allostatic load and cardiovascular disease(44), and between its metabolic and non-metabolic subdomains and the semen outcomes to explore the relative contributions of different biological systems.

In summary, my dissertation is designed to fill specific gaps in the male reproductive health literature. First, my systematic review attempts to make some sense of the mixed results of prior studies of BMI and semen quality by analyzing them according to various features of their sample populations, which earlier reviews have not done. Second, my study of the relationship of birth weight and adiposity to semen quality is the first to test whether adiposity at various critical periods in childhood and adulthood is related to semen quality in midlife and whether there is a cumulative effect over time, questions that have not been able to be asked or answered in semen studies up until now, as they have not had the advantage of a longitudinal data set based on a discrete birth cohort that includes data from middle age. Finally, my study of allostatic load advances our understanding of how the embodiment of stress over time affects the three different semen parameters, in contrast to prior stress and semen studies that have all been cross-sectional and used external measures of stress.

The broader goal of my dissertation project is to further our understanding of some of the biological mechanisms underlying sperm production, including whether physiologic stress related to adiposity is particularly detrimental at various times in the life course and whether particular systems of the body—metabolic vs. immune, for example—have stronger or weaker relationships to different semen parameters. In terms of public health, a better understanding of predictors of poor semen quality may ultimately help us to reduce the apparent decline in semen quality seen in many parts of the world that contributes to an increasing reliance on ART. It also may help us to provide early intervention to mitigate the adverse health outcomes in men that may be foreshadowed by poor semen quality.

CHAPTER 1. THE RELATIONSHIP BETWEEN ADULT BODY MASS INDEX AND SPERM CONCENTRATION, MOTILITY, AND MORPHOLOGY: A SYSTEMATIC LITERATURE REVIEW

1.1 ABSTRACT

Background

Numerous studies have investigated the relationship between body mass index (BMI) and semen quality, but results have been inconclusive.

Objectives

The objective of this study is to systematically review the literature on BMI and the three main semen parameters used to diagnose male infertility: sperm concentration, motility, and morphology.

Data sources

PubMed and EMBASE were searched using a combination of keywords and medical subject heading terms relating to adiposity and semen quality, and Web of Science was used to cull additional papers that referenced the six most frequently cited articles.

Study eligibility criteria and participants

All observational epidemiologic studies published in English in peer-reviewed journals through August 2, 2016, that reported a statistical test of the relationship between BMI and any of the three semen parameters were eligible for inclusion except for those conducted in the context of cancer research, urogenital disorders, or outcomes of assisted reproduction. Eligible study populations included those sampled from fertility clinics or andrology labs, from populations with proven fertility, from the general population, and from a combination of different sources.

Study appraisal and synthesis methods

Titles and abstracts were screened in two rounds. Articles deemed eligible were then reviewed in full by the lead author, who abstracted data from the 56 papers that met all inclusion criteria. Results were summarized by semen outcome measure; stratified by study size, source population, participant age, whether or not they controlled for key covariates, and their percentage of overweight or obese participants (BMI ≥25 kg/m^2); and mapped by study site to assess geographic patterns.

Results

The preponderance of evidence does not support a linear association between BMI and any of the semen outcomes. However, evidence suggests a likely inverse U-shaped relationship between BMI and sperm concentration and potentially a threshold effect in which men in the highest BMI categories have reduced percent motility and normal morphology.

Limitations

Although 664 records were reviewed, there is a chance that some were missed or eliminated in error. Despite stratifying results by various criteria, there was still substantial heterogeneity among studies based on cohort composition and analysis techniques that precluded definitive conclusions.

Conclusions and implications of key findings

Under- and overweight men have lower sperm concentration than men of normal weight, and there may be a negative association between BMI and percent motility and normal morphology at the high end of the BMI distribution. Latitude—an indicator of climate and light--and the presence of environmental toxicants may moderate the effect of BMI on semen quality.

1.2 INTRODUCTION

Ecological studies that depict a gradual decline in sperm concentration since the 1930s(21), while controversial when originally published, have been replicated in several Westernized countries(45, 46) but with regional variation(47), suggesting the possible involvement of environmental exposures on sperm production. While chemical toxicants associated with industrialization such as persistent organic pollutants (POPs) (reviewed in (48)), phthalates (reviewed in (49)), and those contained in air pollution(50) have been implicated in this decline, another potential contributor is body mass index (BMI), which has been increasing concurrently with observed decreases in sperm concentration and in similar geographic regions(51).

Numerous observational studies have examined the relationship between BMI and semen quality, but findings have been conflicting. While inconsistent covariate control may account for some of the discrepancy in results (some studies do not adjust for any; others adjust for different sets of covariates), the major source of noncomparability between studies that could explain their contradictory conclusions is heterogeneity among the populations sampled. Some studies sampled men from the general population while others recruited from fertility clinics and still others included only men of proven fertility. Some studies included men of all ages while others exclusively sampled young men (generally students or military recruits). Furthermore, studies were conducted in various regions, among populations with different racial and ethnic compositions, and in environmental contexts that differed according to both natural and chemical exposures. Any of these factors (fertility status, age, race/ethnicity, environmental conditions and exposures) could potentially interact with BMI to influence semen quality, thereby explaining the lack of consistent results among studies. In addition, the degree to which any of these factors are associated with the BMI distribution in a given study sample may influence its results. For example, if BMI is not associated with sperm concentration among men in the normal BMI range, but negatively associated with sperm concentration among men who are overweight or obese, analyses performed in cohorts comprising only young men, who generally have a BMI distribution that includes fewer subjects with high BMI than cohorts comprising men of all ages, will be less likely to detect associations driven by subjects in the overweight or obese category.

Despite this heterogeneity, three summary analyses of the relationship between BMI and semen quality have been conducted in recent years. MacDonald et al. performed both a systematic review and analysis, published in 2010. Their qualitative review of 13 studies of BMI and semen parameters(38) reported mixed results. Among the 10 studies that considered the relationship between BMI and sperm concentration, one found a positive association, while the rest found negative or no associations. One of 4 studies that analyzed the relationship between BMI and morphology found a higher percentage of abnormal morphology among obese men vs. non-obese men, but the others found no association. None of the 5 studies that reported on BMI and motility found an association. For their analysis, they included only 5 studies (4,853 men in total) that reported mean or median total sperm count or concentration by BMI category. Mean or median values for each BMI category were extracted and weighted by the number of subjects in the BMI group. Using linear models clustered by study in which BMI category was considered as an ordinal variable, they concluded that BMI category was not associated with mean or median sperm concentration, mean or median total sperm count, semen volume, or average sperm motility. Their analysis of mean sperm concentration combined data from 4 studies that differed substantially in source populations: 2 large multisite studies that recruited men from the general population, one from Europe and one from China; and 2 small studies that recruited men from fertility clinics in Hungary. Their analysis of median sperm concentration used data from only 2 studies, both large and drawn from the general population, although one recruited men of various ages while the other included only young military recruits. Analyses of mean and median total sperm count also used data from only 2 studies each; data from 3 studies were used in the volume and motility analyses. Although their data were organized by BMI category, they did not test for nonlinear relationships.

An updated collaborative meta-analysis by Sermondade et al. published in 2013(39) combined data from 21 studies (13,077 men in total) to assess the relationship between BMI, modeled categorically with BMI ≥18.5 and ≤24.9 kg/m^2 as the reference group, and categories of total sperm count (azoospermia, oligozoospermia, and normozoospermia—no, low, or normal sperm count). Using random effects models in which studies were weighted by statistical size, they found a J-shaped association, with underweight men more likely to have azoospermia or oligozoospermia (odds ratio (OR) = 1.46, 95% confidence interval (CI) [1.14, 1.88]) compared to normal weight men, and an increasing dose-response

relationship between BMI and azoospermia or oligozoospermia among overweight, obese, and morbidly obese men (OR = 1.06, 95% CI [0.95, 1.18], OR = 1.31, 95% CI [1.07, 1.61], and OR = 1.97, 95% CI [1.27, 3.07], respectively) compared to normal weight men. The same J-shaped relationship was found when comparing odds of low sperm concentration (<15 million sperm/mL) across BMI categories. Results were comparable when stratified by study population (general vs. fertility clinic).

In a meta-analysis published in 2015(40), Campbell et al. combined data from 17 studies that examined BMI category and sperm concentration, 13 conducted among men recruited from fertility clinics and 4 among men from the general population. Using a random effects model, they found no differences in mean concentration among BMI categories when the two groups were analyzed together or separately. Among the 12 studies that looked at BMI category and progressive motility, they found a small but statistically significant decrease in percent progressive motility among obese men compared to men with normal BMI (weighted mean difference (WMD) = -3.72%, 95% CI [−7.11, -0.33]). When the 9 clinical studies and 3 general population studies were analyzed separately, results were in the same direction but not statistically significant. Campbell et al. also found that obese men had significantly reduced percent normal morphology compared to men with normal BMI among 5 studies conducted in fertility clinic populations that used WHO criteria for classifying morphologically abnormal sperm (WMD = -2.08%, 95% CI [-3.25, -0.92]), but the relationship was no longer significant when the 2 clinical studies that used an alternative method, Kruger's strict criteria, were added to the analysis.

The inconsistent conclusions presented in these three papers reflect important methodological differences. Each focused on different semen quality outcomes and had different data formatting requirements for inclusion, so that only two studies were included in all three analyses. While MacDonald et al. chose to test only the significance of their linear regression coefficients and found no association between BMI and sperm concentration, Sermondade et al. found evidence of a J-shaped relationship with low sperm concentration and Campbell et al. found evidence of a negative association between obesity and percent motility and normal morphology when they compared means across BMI levels. All three groups appropriately dealt with heterogeneity across studies by using weighted linear regression and clustering or using random effects models, but none of them adjusted for any covariates in their models. MacDonald et al. did not perform any subgroup analyses, and Sermondade et al. and Campbell et al.

stratified by source population, but did not consider other potential sources of noncomparability that might bias their results.

The objective of this qualitative review is to summarize findings in the published literature relating to the relationship between BMI and the three semen parameters commonly used to diagnose male infertility(52)--sperm concentration, motility, and morphology—and assess whether any variability in results may be traced to differences in the fertility status of the source population, participant ages, study size, study quality, sample distribution of BMI, or geographic region.

1.3 METHODS

1.3.1 Design

We conducted a systematic review and qualitative analysis of the published literature in accordance with the relevant Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines(53) (see Supplemental Table 1.1 for PRISMA checklist).

1.3.2 Search Strategy

We initially searched PubMed and EMBASE using a combination of keywords and medical subject heading terms relating to birth weight, adiposity, and semen quality, e.g., “sperm count,” “sperm motility,” “spermatozoon density,” and “semen parameters” (Supplemental Table 1.2). We then searched Web of Science for additional references that cited the six most frequently referenced articles on BMI and semen quality(38, 39, 54-57). Finally, we included any remaining articles mentioned in the three meta-analyses described above(38-40) and one critical review of the literature(56). After duplicates were removed, our search yielded 664 distinct records.

1.3.3 Eligibility Criteria

In order to be as inclusive as possible, our review included all original observational studies that provided a statistical test of the relationship between adult BMI and one or more of three specified semen

quality outcomes: sperm concentration, percent motile sperm, and percent normal/abnormal morphology. The test may have constituted the study's primary analysis or may have been conducted as part of preliminary bivariate analyses in cases when the main aim of the paper was to assess a different relationship. Eligible study populations included those sampled from fertility clinics or andrology labs who were having semen evaluations as part of an infertility workup, those sampled from populations with proven fertility, those sampled from the general population or subgroups whose fertility status was unknown, and those sampled from a combination of sources. Non-human studies, review articles, commentaries, editorials, case reports, clinical trials, and studies focused on semen quality in the context of cancer treatment, urogenital disorders, or ART outcomes were excluded. All English-language studies meeting these criteria that were accepted for publication in a peer-reviewed journal and publicly available through August 2, 2016, were considered for this review.

1.3.4 Screening and Study Selection

The titles and abstracts of retrieved articles were first screened by the primary investigator (LGK) in EndNote X7 to weed out those that clearly met predefined exclusion criteria. The remaining references were uploaded to Covidence where their abstracts were screened independently by two investigators (LGK and Elizabeth Widen) to assess whether they met both inclusion and exclusion criteria (Supplemental Table 1.2). Any disagreement or uncertainty as to the eligibility of an article was resolved through discussion. The full texts of all retained articles were reviewed by LGK, who eliminated those that did not meet the specified exposure and outcome definitions.

1.3.5 Data Collection

Information was extracted from each study and entered into an Excel spreadsheet under the following headlines: first author, date, title, study design, location, sample size, age, BMI distribution, source population, inclusion/exclusion criteria, exposure measure, outcome measure(s), covariates, and results for each semen outcome. Study findings were described and categorized as "no association," "negative association," or "positive association" if they reported a statistically significant test for

correlation, linear trend, or differences between groups. They were categorized as “non-linear” if they reported a U- or inverse U-shaped association. We based our categorizations on adjusted results when both unadjusted and adjusted were reported. Additional information was noted regarding each study’s design and analysis, including whether or not the authors used measured or self-reported height and weight to calculate BMI, followed a WHO semen analysis protocol, appropriately handled outcomes that were not normally distributed, and adjusted or restricted for age and length of abstinence since last ejaculation. This last criterion, one of several included in a proposed checklist for acceptable studies based on human semen analyses(58) (others were difficult to assess retrospectively or did not apply to analyses that were performed under earlier WHO protocols), was designated the marker of a high-quality analysis. Studies that included only young men were considered to be restricted for age; studies that accepted only semen samples provided within approximately 2-7 days of sexual abstinence were considered to be restricted for abstinence.

1.3.6 Synthesis of results

Results for the relationship between BMI and each of the three semen parameters were analyzed separately. In addition to considering the findings of all of the included studies, we compared subgroups, stratified by study size, quality, source population, participant age, and percentage of overweight or obese participants (BMI ≥25 kg/m^2). We also mapped the results of the studies to ascertain if the results followed a geographical pattern using BatchGeo (BatchGeo LLC, Albany, NY). When a study was conducted in multiple locations, the sites were mapped separately if the authors confirmed that the results were the same across locations(54, 59, 60); otherwise, results were represented by a single marker at the first location listed by the authors.

1.4 RESULTS

1.4.1 Study Selection

Our initial search garnered 664 unique references, 491 of which were eliminated upon initial screening of titles and abstracts by LGK. Of the 173 abstracts reviewed by both LGK and EW, 77 were

considered eligible according to predetermined inclusion and exclusion criteria. LGK reviewed the full texts and eliminated an additional 21 papers that did not meet the specified exposure or outcome measures or duplicated another study's cohort, yielding 56 articles for inclusion (Figure 1.1).

1.4.2 Study characteristics

Characteristics of the 56 studies included in our analysis are presented in Table 1.1. Fifty-five (98%) tested an association between BMI and sperm concentration, 50 (89%) tested an association between BMI and percent motile sperm, and 41 (73%) tested an association between BMI and percent normal/abnormal morphology. Thirty-three studies (59%) drew subjects from fertility clinics or andrology labs, 2 studies (4%) recruited participants with proven fertility (partners of pregnant women), 17 studies (30%) included men from the general population or from subsamples of unknown fertility status (e.g., occupational cohorts), and 4 studies (7%) drew from a combination of sources. Five studies (9%) were restricted to young men, generally military recruits or students, ranging in age from 18 to 23, while the rest included men of mixed ages, overall ranging from 16 to 75 years. Sample sizes ranged from 42 to 10,197. Of the 47 studies (84%) that reported the distribution of participants' BMI, 9 (19%) enrolled <30% overweight or obese men and 19 (40%) enrolled >60% overweight or obese men (the latter group included 4 studies that recruited based on overweight/obesity status). Twenty-nine (52%) studies did not control for any covariates. The analyses presented in the remaining 27 studies (48%) were considered high quality, as they included, at minimum, age and abstinence time among the variables controlled for through restriction or regression.

Studies were conducted in 39 different countries and on every continent except Antarctica. All analyses were cross-sectional, although in three cases the analyses were based on data abstracted from prospective cohort studies (54, 55, 57, 59-111).

1.4.3 Results of individual studies

1.4.3.1 Concentration

Table 1.2a summarizes our findings regarding sperm concentration. Among the 55 studies that tested the relationship between BMI and sperm concentration, 33 found no statistically significant association, 20 found a negative association, 1 found a positive association, and 1 found an inverse U-shaped association. Fertility status did not appear to be related to the type of association reported: 57.6% of studies that found no association were conducted in a fertility clinic/andrology lab compared to 65.0% of those that found a negative association, and 30.3% of studies that found no association drew from the general population compared 25.0% of those that found a negative association. The two studies conducted among men of proven fertility both reported no association. All but one of the studies conducted among young men reported no association. Studies that found no association were more likely to be large (>600 participants) than those that found a negative association (42.4% vs. 20.0%) and less likely to be small (<100 participants; 12.1% vs. 35.0%), suggesting that limited sample size did not necessarily preclude the finding of statistically significant associations among the studies analyzed. The prevalence of overweight or obesity in the study samples appeared to be related to the type of association found: 27.3% of those that found no association had >60% overweight/obese participants compared to 50.0% those that found a negative association, while 45.5% of those that found no association had 30-60% overweight/obese participants compared to 15.0% of those that found a negative association. The one study to find a positive association had <30% overweight/obese participants. Finally, a greater percentage of studies reporting no association controlled for both age and abstinence in their analyses compared with those that found a negative association (51.5% vs. 40.0%), as did the study that found a positive association.

Among studies that controlled for both age and abstinence (high-quality analyses) were 17 that found no association, 8 that found a negative association, as well as the single study that found a positive association. Once again, study results differed according to the prevalence of overweight or obesity in the study populations: 35.3% of those that found no association enrolled >60% overweight/obese participants compared to 62.5% of those that found a negative association, while 35.3% of studies that found no association enrolled 30-60% overweight/obese participants compared to 12.5% of studies that found a

negative association. More studies that found no association were large compared to those that found a negative association (52.9% vs. 37.5%). Both of the small studies found a negative association, likely due to the fact that both of the small studies enrolled high percentages of overweight or obese men (in fact, one recruited exclusively obese men(77)) rather than to any inherent difference in their methods. The single study that reported a positive association was large, included a mixed-age sample from the general population, and had a low percentage of overweight or obese participants.

One large study with high-quality analyses, conducted by Jensen et al., reported an inverse U-shaped association between BMI and sperm concentration. Those who were underweight and overweight had lower mean sperm concentration compared to those who had normal BMI (b_{adj} = -28.1, 95% CI [-47.9, -8.3] and b_{adj} = -21.6, 95% CI [-39.4, -4.0], respectively). Notably, this study exclusively enrolled young military recruits, and only 19% of the sample had BMI ≥25 kg/m^2(57). Lu et al. also observed that sperm concentration in underweight and obese men was lower than in normal weight men, but the differences were not statistically significant. Although they did not report details of their BMI distribution, the mean BMI in their sample was 23.9 kg/m^2, which is one of the lowest reported among studies that included mixed-age samples(112). In both cases, the high percentage of men at the low end of the BMI range may have made it possible ascertain a difference between underweight and normal weight men that would not have been detectable in a cohort of men with higher BMIs.

When the results of all of the concentration studies were mapped, no obvious pattern emerged (Supplemental Figure 1.1a). When only the sites of the studies with high-quality analyses were mapped, it appeared that while the majority reported no association between BMI and sperm concentration, 4 of the 8 studies that reported a negative association were clustered in northwestern Europe, between Paris and Norway(57, 62, 65, 77, 79). Two others were located in Northeastern/Midwestern US and Canada (multisite, results not reported separately)(66) and in northwestern Russia(74) (Figure 1.2a).

1.4.3.2 Motility

Table 1.2b summarizes results from the 50 studies that analyzed the relationship between BMI and percent sperm motility. Thirty-six found no statistically significant association, 12 found a negative association, 1 found a positive association, and one found a U-shaped association. As with concentration,

fertility status did not appear to be related to study results: 63.9% of studies that reported no association drew from fertility clinics/andrology labs compared to 58.3% of those that reported a negative association and 27.8% of those that reported no association drew from the general population compared to 25.0% of those that reported a negative association. Both studies that included men known to be fertile reported no association. All of the studies conducted among young men reported no association. The sample sizes of studies were similarly distributed between those that found no association and those that found a negative association. Among those that reported no association, 27.8% were conducted among samples with a high percentage of overweight/obese compared to 66.7% of studies that reported a negative association, but the situation was reversed for studies in which 30-60% of participants were overweight/obese: 41.7% of studies reporting no association vs. 16.7% of studies reporting a negative association. Nineteen (52.8%) of the studies that found no association, six (50.0%) of the studies that found a negative association, and the study that found a positive association controlled for both age and abstinence; therefore, their analyses were considered to be high quality.

Among the studies presenting high-quality analyses, a higher percentage of those reporting no association compared to a negative association recruited from the general population (47.4% vs. 16.7%) and a lower percentage drew from fertility clinics/andrology labs (42.1% vs. 66.7%). None of the studies that found a negative association enrolled <30% overweight/obese, while two-thirds of studies that found a negative association included >60% overweight/obese participants. The single study that reported a positive association was large, included men of mixed ages from the general population, and enrolled 30-60% overweight/obese participants.

In contrast to their non-statistically significant inverse U-shaped finding for sperm concentration, Lu et al. found a statistically significant U-shaped association between BMI and percent progressive motility in unadjusted analyses, with men in the highest and lowest BMI categories having higher motility compared to those in the normal BMI range(87). Bandel et al. also found that those in the obese category had higher motility compared to normal weight men; they may not have had enough men at the low end of the BMI range to detect a difference between underweight and normal weight men.

In both the map including all of the motility studies and the map including only the high-quality analyses, it was notable that all 4 sites that reported a positive association (representing Bandel et al.'s

multisite study in which the results were the same across sites(54, 59)) were located in Europe, and included the three northernmost locations of any studies: two in Greenland and one in upper Norway (Supplemental Figure 1.1b, Figure 1.2b).

1.4.3.3 Morphology

Of the 41 studies that examined morphology, summarized in Table 1.2c, 31 found no statistically significant association between BMI and percent normal morphology, 8 found a negative association, and 2 found a positive association. Studies that found no association were more likely than studies that found a negative association to be conducted among participants drawn from fertility clinics/andrology labs (67.7% vs. 25.0%) and less likely to be conducted among participants from the general population (19.4% vs. 50.0%). Both studies conducted among men of proven fertility and all four studies that included exclusively young men found no association. Among studies that found no association, 32.3% had large sample sizes compared to 50.0% of studies that found negative associations. Among studies that found no association, 32.3% included >60% overweight/obese participants compared to 62.5% of those that found a negative association. Fifteen (48.4%) of the studies that found no association, five (62.5%) of the studies that found a negative association, and both of the studies that found a positive association controlled for both age and abstinence.

Among the studies presenting high-quality analyses, a greater percentage of those that found no association recruited from fertility clinics/andrology labs compared to those that found a negative association (53.3% vs. 40.0%). A smaller percentage of those that found no association had a large sample size (46.7% vs. 60.0%) and >60% overweight/obese participants (33.3% vs. 60.0%) compared to those that found a negative association. The two studies that reported a positive association were conducted among men of mixed age; one was large and drew from the general population while the other was medium-sized and recruited subjects from fertility clinics.

When the results of all of the morphology studies were mapped, it became apparent that with the exception of a study conducted in New Zealand, which reported a positive association(88), all of the studies conducted in the southern hemisphere reported no association (Supplemental Figure 1.1c). When results of the high-quality analyses were mapped, all but one of the studies that found a negative

association were located in cold climates (Northeastern/Midwestern US and Canada (multisite, results not reported separately)(66), and northern Europe(62, 77, 102)) (Figure 1.2c).

1.5 DISCUSSION

1.5.1 Summary of evidence

Although the majority of studies reported no statistically significant association between BMI and sperm concentration, motility, or morphology, our review noted some trends that might suggest variations in these relationships based on characteristics of the study populations. In particular, sample BMI distribution and study location appear to be related to the direction of some of the associations.

Not all studies reported the breakdown of their populations by BMI category, but among those that did, studies with high-quality analyses (controlling for both age and ejaculatory abstinence time) that enrolled high percentages (>60%) of overweight/obese participants were more likely to report negative associations between BMI and sperm concentration compared to those with 30-60% overweight/obese participants. These associations were driven by those in the overweight and/or obese categories, who in many cases had significantly lower mean sperm concentration compared to those in the normal category(64, 65, 74, 79, 106). By contrast, Qin et al., the only group to report a positive association, had a low percentage of overweight or obese participants (26.1%) and high percentage of normal or underweight participants (73.9%), with an overall mean sample BMI of 23.2 ± 2.9 kg/m^2(95). Similarly, Jensen et al., whose sample was young and included few overweight or obese participants, noted that those with normal BMI had higher sperm concentration than those who were underweight. These findings are in keeping with Sermondade et al.'s meta-analysis that demonstrated a nonlinear association in which those in the normal BMI group (18.5 kg/m^2 ≤BMI ≤24.9 kg/m^2) had reduced odds of low sperm concentration compared to those in the underweight (BMI <18.5 kg/m^2) and overweight/obese groups (≥25 kg/m^2).

Negative associations between BMI and motility found in high-quality analyses tended to be driven by those at the highest end of the BMI range. Andersen et al. reported that those who were morbidly obese had lower percent motility compared to those who were of normal weight(62), while

Bakos et al. found those who were morbidly obese had significantly lower percent motility compared to those who were obese, overweight, and normal weight(64). Among studies that reported a negative association between BMI and percent normal morphology, once again, the relationship was most apparent when contrasting those who were obese or morbidly obese to those who were normal weight(62, 84, 100, 102). As with concentration, Qin et al. found a positive association between BMI and percent normal morphology that was driven by differences between those who were underweight and those who were normal weight or overweight, which they were likely able to detect because of the relatively low BMI distribution in their sample(95). These findings suggest that the relationship between BMI and sperm concentration—and possibly motility and morphology--may not be linear and that there may be BMI thresholds above and below which negative effects may be seen. They also highlight the importance of insuring adequate sample size at the tail ends of the sample BMI distribution so that analyses are adequately powered to detect nonlinear associations.

Much has been written about possible pathways linking obesity to reduced testosterone and, consequently, impaired sperm production(56, 113, 114). Obesity is associated with a constellation of hormonal dysregulation in men known as hyperestrogenic hypogonadotropic hypoandrogenemia: in the presence of excess adipose tissue, adrenal and testicular androgens are aromatized into estrogens, which suppress release of gonadotropins from the pituitary; with reduced hormonal stimulation, the Leydig cells in the testes produce less testosterone(113). In addition to aromatase, white adipose tissue produces leptin, which also downregulates testosterone production, and resistin, which is hypothesized to induce insulin resistance(114). High circulating insulin is associated with low total and free testosterone; there is some debate as to whether this relationship is mediated by sex hormone binding globulin. Overweight men are also at increased risk of sleep apnea, and both poor sleep quality and shortened sleep duration are risk factors for low testosterone(113). Increased adipose tissue in the genital area, especially in conjunction with sedentary behavior, can lead to higher scrotal temperature, which has been associated with reduced sperm concentration(56, 114). Motility may also be deleteriously affected by obesity, as insulin resistance is associated with increased levels of reactive oxygen species (free radicals that contain oxygen), which can damage sperm mitochondria(114). Reactive oxygen species also weaken the lipid structure of cell membranes, and to the degree to which the membranes of Sertoli cells, which

nurture developing spermatocytes, are involved in spermatid head-shaping and tail production, obesity may additionally affect sperm morphology(115). There is mounting evidence that POPs, which accumulate in fatty tissue, may impair semen quality(48, 116). Finally, recent research has identified a number of genes that are associated with both obesity and male infertility, raising the possibility that links between BMI and semen quality may result from a common genetic cause(117).

Far less is known about the effects of underweight on semen quality. A case study that followed three male patients with acute anorexia nervosa through therapeutic weight gain provides provocative evidence that the hormonal profile of men with extremely low BMI may be similar to that of men with high BMI. Leptin, testosterone, and gonadotropin levels were all low at intake and increased with weight gain, and the change in leptin was positively correlated with changes in testosterone, luteinizing hormone, and follicle stimulating hormone (Pearson correlation coefficients (p-values): 0.55 (0.002), 0.46 (0.01), 0.81 (0.0001), respectively)(118).

The geographical distribution of the studies' results, although not a source of statistical evidence, suggest two potential ways in which environmental factors may influence the relationship between obesity and semen quality. First, the clustering of all but two of the high-quality sites at which a negative association was found between BMI and sperm concentration, all of the high-quality sites at which a positive association was found between BMI and motility, and all but 1 of the high-quality sites where a negative association was found between BMI and morphology in northern Europe and northern North America may indicate potential moderation by factors associated with high latitude, such as climate and light. Second, 4 out of the 8 studies that found a negative association between BMI and sperm concentration were located in countries with high incidence rates of testicular cancer, including 2 studies conducted in Norway and Denmark, which have the highest age-standardized rates in the world (12.7 and 12.5 per 100,000, respectively, in 2012)(119). Skakkebaek et al. have postulated that the coincident increase in testicular cancer, cryptorchidism, hypospadias, and poor semen quality in industrialized Western countries, a collection of urogenital conditions they refer to as testicular dysgenesis syndrome, may result from prenatal exposure to endocrine disrupters such as POPs(120). Several of these compounds have been associated with increased obesity in longitudinal studies of prenatal exposure to POPs and infant growth(121, 122) and early childhood adiposity(123), as well as adult dietary intake of

food containing polychlorinated biphenyls and subsequent development of obesity(124). A cross-sectional study using data from the National Health and Nutrition Examination Survey showed POPs to be more strongly associated with metabolically active trunk fat than leg fat(125). If confirmed, these findings would imply that in regions where POPs are prevalent, obesity and poor semen quality may not be causally related, but associated through a common environmental cause.

1.5.2 Limitations

Although 664 records were reviewed, there is a chance that some were missed or eliminated in error, which might lead to selection bias. Despite stratifying results by various criteria in order to reduce some of the heterogeneity among studies that plagues systematic reviews, there remained substantial differences among studies that precluded definitive conclusions.

1.5.3 Strengths

Because we included studies in which the relationship between BMI and semen outcome was not necessarily the main effect, we were able to include far more studies than the three prior published reviews. We also considered all three of the semen quality measures most commonly used to diagnose male infertility. While Campbell et al. stratified their results by fertility status, neither they nor the other two groups considered any of the other potential differences we identified among sample populations that might influence study findings.

1.5.4 Conclusion

The preponderance of evidence does not support a linear association between BMI and any of the semen outcomes: sperm concentration, motility or morphology. Results were similar when stratified by age, study population, study size, or quality. There is likely an inverse U-shaped relationship between BMI and sperm concentration, however, in which under- and overweight men have lower sperm concentration compared to normal weight men, and potentially a threshold effect in which men in the highest BMI categories have reduced percent motility and normal morphology. Future studies should explore potential

interactions between BMI and the physical environment, especially climate and endocrine disrupting POPs.

Table 1.1. Characteristics and results of studies investigating BMI and semen quality.

First author, publication date	Location	N	Source population	Age (years)	BMI (kg/m2)	High quality	Conc	Mot	Morp
Aggerholm, 2008	Aarhus, Copehnagen, Jutland, Denmark; Belgium; England; Italy; Sweden; Greenland; Kharkiv, Ukraine; Warsaw, Poland	2,139	5 occupational studies: PLANNERS (1992-5) Denmark, trades-union members with no children; GARDENERS (1994) Denmark, flower greenhouse workers; FARMERS (1995-6) Denmark, organic and traditional farmers; ASCLEPIOS (1996-7) Belgium, England, Italy, workers with and without lead exposure; INUENDO (2002-4) Greenland, Sweden, Ukraine, Poland, partners of pregnant women with contrasting blood levels of POPs	Range 18-66	47% >25, 8.2% >30	Y	NA	NA	
Alshahrani, 2016	Alkharj, Saudi Arabia	439	Male partners of infertile couples who presented for evaluation between December 2013 and May 2015 whose female partners had no apparent reason for infertility	Mean 36.85 (SD 6.73)	Mean 29.67 (SD 5.89); 82.9% >=25, 42.1% >30	N	Neg: r=-0.101, p=0.035	NA	NA
Andersen, 2015	Oslo, Tonsberg, Porsgrunn, Norway	166	Between 2008 and 2013, overweight/obese men recruited by newspaper ads, public notices, commercial weight loss programs, 2 public obesity clinics; normal weight men recruited by newspaper ads and from a fertility clinic because of female-factor infertility	Range 22-61	73% >=25, 41.6% >=30	Y	Neg: b=-0.088 [-0.153, -0.023] across categories, p=0.009	Neg: b=-0.675 [-1.156, -0.194] across categories, p=0.007; morbidly obese <normal, p=0.001	Neg: b=-0.078 [-0.124, -0.032] across categories, p=0.001; morbidly obese <normal, p<0.001

Anifandis, 2012	Larisa, Greece	301	Male partners of couples seeking infertility treatment, 2010-2011	Range of means among BMI groups 35.7-38.7	73.4% >25	N	NA	NA	
Bakos, 2011	Australia	305	Male partners of couples undergoing fresh ART cycles between January and May 2008	Mean 35.5 (SEM 0.7)	79.3% >=25, 30.8% >30	Y*	Neg: overweight, obese, morbidly obese <normal p<0.05	Neg: morbidly obese <obese, overweight, normal, p<0.05	NA
Bandel, 2015	Sweden; Greenland; Kharkiv, Ukraine; Warsaw, Poland; Tromsoe, Oslo, Norway	1,503	4 cohorts: Swedish military conscripts 2000-2001, Swedish military conscripts 2008-2010, INUENDO (see Aggerholm), Norwegian men recruited through ads 2001-2 age 19-40	Mean 27.9 Range 17.5-69	Median 24.0 Range 11.5-57.8; 38% >=25	Y	NA	Pos: obese >normal, mean difference 1.15% [1.02-1.30%], p<0.05	
Belloc, 2014	Paris, France	10,197	All men referred to lab for semen evaluation from October 9, 2010-October 8, 2011	Mean 37.1 Range 17-72	Mean 25.0 (SD 3.4); 42.9% >=25, 7.5% >=30	Y	Neg: overweight, obese, morbidly obese <normal, p=0.05, 0.001, 0.03	Neg: overweight <normal, p=0.01	NA
Bieniek, 2016	USA, Canada	4,440	All men referred for male infertility to three North American clinics from 2002-2014	Mean 36.1 (SD 7.6)	Mean 27.6; 68.4% >=25, 23.3% >30	Y*	Neg: r=-0.08, p<0.001	NA	Neg: r=-0.04, p=0.015
Chavarro, 2010	Boston, MA, USA	483	Male partners of couples seeking infertility treatment, 2000-2006	Mean 36.3 (SD 5.4)	74.5 >=25, 26.3% >=30	Y	NA	NA	NA
Duits, 2010	Netherlands	1,401	Male partners of couples seeking infertility treatment, January 2000-January 2007	Mean 36.4 (SD 6.5)	52.3% >25, 10.4% >30	Y*	NA	NA	NA

Egwurugwu, 2011	Orlu, Nigeria	109	Male patients presenting to fertility clinic for evaluation between January 1 and December 31, 2009	Mean 28.3 (SEM 0.76) Range 20-50	Mean 25.53 (SEM 0.29)	N	Neg: r=-0.273, p<0.01	NA	
Ehala-Aleksejev, 2015	Tartu, Tallinn, Estonia	260	Male partners of pregnant women at a university hospital recruited 2010-2011	32.3 (SD 6.7) Range 21-57	51.2% >=25, 14.6% >=30	Y	NA	NA	NA
Eisenberg, 2014	Texas, Michigan	468	Male partners of couples trying to conceive, 2005-9	Mean 31.8 (SD 4.8)	Mean 29.8 (SD 5.6); 82.3% >=25, 41.4% >=30	Y*	NA		NA
Eskandar, 2012	Abha, Saudi Arabia	500	Male partners of couples seeking infertility treatment, February 2009-February 2011	Mean 34.77 (SD 7.67) Range 21-68	Mean 28.12 (SD 6.1); 35.6% >=30	N	NA	NA	NA
Fariello, 2012	Sao Paulo, Brazil	305	Male patients presenting to fertility clinic for evaluation	Mean normal 33.5 (SD 6.1), overweight 34.7 (SD 7.9), obese 34.3 (SD 4.9)	73.1% >=25, 11.8% >=30	N	Neg: obese <normal, p=0.078	Neg: overweight, obese <normal, p=0.001	NA
Fejes, 2006	Szeged, Hungary	42	Oligospermatic men who presented to infertility clinic	Mean 28.2 (SD 5.3)	Mean 27.6 (SD 4.6); 59.5% >25	N	Neg: overweight/ obese < underweight/ normal, p<0.05	NA	NA
Gutorova, 2014	Arkhangelsk, Russia	99	Volunteer men from northern Russia with Polar adaptive metabolic type	Mean 37.9 (SEM 0.24), Range 23-58	63.6% >=25, 19.2% >=30	Y	Neg: overweight <normal, p<0.05	NA	
Hadjkacem-Loukil, 2015	Tunisia	98	Men recruited from research unit of a hospital between 2001 and 2010	Range 16-51	Mean 22.82 (SD 2.98); 21.4% >=25, 5.1% >=30	N	NA		

Hajshafiha, 2013	Urmia, Iran	159	Male partners of couples seeking infertility treatment	Not specified	53.4% >25, 11.9% >30	N	NA	NA	NA
Hakonsen, 2011	Ebeltoft, Denmark	43	Participants in 14-week residential weight-loss program, April 2006-April 2009	Median 32 Range 20-59	Median 44 Range 33-61; All >33	Y	Neg: p-trend =0.02	Neg: p-trend =0.04	Neg: p-trend =0.005
Hammiche, 2011	Netherlands	175	Male partners of subfertile couples participating in FOLFO study of food, lifestyle and fertility outcome, recruited between September 2004-January 2007	Median 37 Range 26-59	Median 25.5 Range 18.8-37.9	Y*	NA	NA	NA
Hammiche, 2012	Rotterdam, Netherlands	450	Male partners of couples seeking infertility treatment or preconception counseling from October 2007-October 2010	Median 35; Range: 22-60	Median 26.3 Range 19.1-49.0; 66% >=25, 16% >=30	Y*	Neg: b=-0.042, p=0.07; obese vs. normal b=-0.77, p=0.006	NA	
Hart, 2015	Perth, Western Australia	365	Male members of a population-based birth cohort	Range 20-22	Mean 24.2 (SD 3.9)	Y	NA	NA	NA
Hofny, 2010	Cairo, Egypt	122	Obese men (BMI >30) recruited from an andrology clinic	Mean infertile 29.35 (SD 0.9), fertile: 29.79 (SD 1.1)	All >30	N	Neg: r= -0.412, p<0.01	Neg: r= -0.439, p<0.01	Neg: r= -0.049, p<0.01
Jensen, 2004	Denmark	1,558	Military recruits undergoing their screening physical between June 1996 and March 1998	Mean 19	14% <20, 19% >=25	Y	NL: inverse U-shape; underweight <normal b=-28.1 [-47.9, -8.3]; overweight <normal b=-21.6 [-39.4, -4.0]	NA	NA

Jørgensen, 2016	Copenhagen, Denmark; Tartu, Estonia; Turku, Finland; Leipzig, Hamburg, Germany; Riga, Latvia; Kaunas, Lithuania; Almeria, Spain	8,182	Military recruits in all sites but Spain; college students in Spain	Median 19.1		N	NA		
Jurewicz, 2014	Poland	344	Male partners of couples seeking infertility treatment	Median 32.2 Range 22-57	Mean 27.2 (SD 3.8); 67.2% >=25, 20.6% >=30	Y	NA	NA	NA
Koloszar, 2005	Szeged, Hungary	274	Male partners of couples seeking infertility treatment	Mean 26.3 (SD 5.8) Range 18-46	Mean 27.6 (SD 4.9); 54.5% >25, 21.2% >30, 10.2% <20,	N	Neg: obese <overweight, normal, underweight, p<0.05		
La Vignera, 2012	Catania, Italy	150	Men selected from the general population: 50 normal, 50 overweight, 50 obese	Mean normal 31.5 (SD 1.1), overweight 31.2 (SD 1.2), obese 31.6 (SD 1.7) Range 20-48	66.7% >25, 33.3% >30	N	NA	Neg: overweight, obese <normal, p<0.05	Neg: obese <normal, overweight, p<0.05
Leisegang, 2014	South Africa	42	Men recruited from medical clinics and ads between July 2011 and August 2012	Mean 36.7 (SD 6.7) Range 24-49	Mean 31.1 (SD 6.2); 83.3% >=25, 54.8% >=30	N	Neg: obese <non-obese, p=0.0145	NA	NA

Li, 2009	Chongqing, Southwest China	1,346	Men recruited from general public through family planning clinics, 2007	Range 20-40	Mean 22.4 (SD 2.9)	Y	NA	NA	
Lu	Nanjing, China	1,132	Male partners of couples seeking infertility treatment between August 2012 and February 2014	Mean 29.07 (SD 4.83) Range 18-55	Mean 23.90 (SD 3.01)	N	NA	NL: U-shaped; underweight, obese >normal, p<0.05	NA
Macdonald, 2013	Auckland, New Zealand	511	Male partners of couples seeking treatment in 3 fertility clinics, May 2008-March 2012	Mean 36.8	Median 27.1, 10-90th percentile 22.8-32.9; 72.8% >=25, 23.3% >=30	Y	NA	NA	Pos: b=0.47, p=0.038
Magnusdottir, 2005	Reykjavik, Iceland	72	Male partners of couples seeking infertility treatment at Iceland's only fertility clinic from March 1999-May 2001; 20 from idiopathic subfertility group (normal quality) and 27 from female factor group	Not specified	Median 26.3 Range 19.7-45.6	N	Neg: r=-0.33, p=0.02		
Martini, 2010	Cordoba, Argentina	794	Male partners of couples seeking infertility treatment, 2006-2007	Mean 34.9 (SEM 0.2) Range 20-65	Mean 27.2 (SEM 0.1) 68.4% >25, 19.5% >30	Y	NA	Neg: b=-0.49, p=0.007	NA
Mohamad Al-Ali, 2014	Regensburg, Germany	2,110	Consecutive men attending fertility clinic from 1994-2010	Mean 31.8 (SD 6.6)	Mean 25.3 (SD 3.4); 50% >=25, 9.1% >30	N	NA	NA	NA
Paasch, 2010	Leipzig, Germany	2157	Male partners of couples seeking infertility treatment whose information is stored in Winsperm andrology data base 1999-2005	Mean 30 (SD 8.5) Range 17-67	Mean 25.0 (SEM 0.08); 48.9% >25, 11.4% >30, 4.6% <20	N		NA	

Pärn, 2015	Uppsala, Sweden	62	Male partners of couples seeking infertility treatment between February 2011 and January 2014	Mean 35.2 (SD 5.7)	Mean 25.8 (SD 4.0); 53.2% >25, 12.9% >30	N	NA	NA	
Pauli, 2008	Hershey, PA, USA	87	Known fertile men recruited from Ob/Gyn practice, men of unknown fertility from infertility clinic	Mean fertile 32.4 (SD 5.8), unknown 31.3 (SD 5) Total range: 19-48		N	NA		NA
Qin, 2007	Hebei, Shanxi, Guizhou, Zhejiang, Shandong, China	990	Men recruited from the general population in 5 cities in both south and north China from January 2001 through December 2002; half urban, half rural	Mean 38.9 (SD 9.7) Range 20-60	Mean 23.2 (SD 2.9); 26.1% >=25, 1.7% >=30, 4.2% < 18.5	Y	Pos: underweight <normal, overweight, p<0.01	NA	Pos: underweight <normal, overweight, p<0.01
Ramlau-Hansen, 2010	Denmark	347	Sons of mothers in Health Habits for Two birth cohort recruited during pregnancy between April 1984 and April 1987 followed up between February 2005 and January 2006	Range 18-21	Median 22.7 Range 16.8-34.8; 24.2% >=25, 6.1% >=30, 3.2% <18.5	Y	NA	NA	NA
Relwani, 2011	New York, NY, USA	530	Men who presented to fertility clinic or andrology lab between July 2007 and April 2008	Range 18-50	Mean 28.18 (SD 4.91); 66.7% >25.8, 33.3% >=29.7, 33.3% <25.8	N	NA	NA	NA
Rybar, 2010	Brno, Czech Republic	153	Male partners of couples seeking infertility treatment	Mean 31.5 (SD 6.2) Range 20-61	51.6% >=25, 10.5% > 30	N	NA	NA	NA

Samavat, 2014	Florence, Italy	48	Obese men recruited from bariatric surgery unit from July 1-December 31, 2013; age-matched controls recruited from infertility clinic	Mean obese 39.5 (SD 10.7), lean 39.2 (SD 6.1)	47.9% >36, 52.1% <25	N	NA	NA	NA
Sekhavat, 2010	Iran	852	Men from the general population from both urban and rural settings recruited from October 2004-September 2006	Range 25-50	Mean 24.7 (SD 3.6); 27.2% >=25, 2.2% >=30	N	Neg: overweight, obese <normal, p=0.01	Neg: obese <normal, p<0.01	Neg: obese <normal, p=0.01
Sermondade, 2013	Bondy, France	306	Male partners of couples seeking fertility treatment between January 2005 and April 2012	Mean normal 35.3, overweight 35.9, obese 35.4	48% >=25, 8.8% >=30	N	NA	NA	NA
Shayeb, 2011	Aberdeen, UK	2,035	Male partners of couples seeking infertility treatment 1990-2007	Range of means among BMI groups 30.6 (SD 5.6) to 34.0 (SD 5.8)	57.9% >=25, 13.2% >=30	Y	NA	NA	Neg: overweight/ obese <normal, underweight, p=0.001
Song, 2013	China	53	Male partners of couples seeking infertility treatment from July 2010-August 2011	Not specified	28.6% >=25	N	Neg: among normal, r=-0.38, p<0.01	Neg: among all, r=-0.51, p<0.01; among overweight, r=-0.52, p<0.01	
Tang, 2015	Guangdong, China	1,213	Husbands of pregnant women recruited through the Family Planning Network from October 2010-September 2012	Mean 31.6 (SD 5.2)	Mean 22.4 (SD 2.2); 7.3% >25	Y*	NA	NA	NA

Thomsen, 2014	Skive, Denmark	612	Male partners of couples seeking fertility treatment between April 2002 and December 2003	Range of means among BMI groups 31.7 (SD 5.3) to 32.4 (SD 3.1)	Mean 25.9 (SD 3.5); 55.9% >=25, 12.1% >30	N	NA	NA	
Tsao, 2015	Taiwan	7,630	Men from the general population who participated in a standard medical screening program run by a private company between January 2008 and May 2013	Mean 31.75 Range 18-75	Mean 23.79 (SD 3.30); 25% >=25.62, 25% <21.60	Y*	Neg: highest quartile <lowest quartile, p<0.05	NA	Neg: p-trend <0.001
Tunc, 2011	Adelaide, Australia	81	Male partners of couples seeking infertility treatment	Not specified		N	Neg: r=-0.33, p=0.002	NA	NA
Umul, 2014	Isparta, Turkey	155	Male partners of couples seeking infertility treatment	Range of means among BMI groups 32.5-35.6	66.5%>=25, 18.1% >=30	N	Neg: overweight <normal, p=0.02; obese <normal, p=0.03	Neg: obese <normal, p=0.02; obese <overweight, p=0.03	NA
Wen-Hao, 2015	China	617	Male partners of couples seeking infertility treatment between August 2011 and July 2013	Mean 32 (SD 5.2) Range 21-67	45.9% >=25, 10.2% >=30	Y	NA	Neg: b=-0.315, p<0.05	NA
Wogatzky, 2012	Bregenz, Austria	1,683	Male partners of couples seeking infertility treatment	Mean 40.4 (SD 5.9) Range 25-72	Mean 26. 1 (SD 3.4); 58.8% >25	N	NA	NA	NA
Yang, 2015	Chongqing, China	796	College students	Median 20 5-95% Range 18-23	13.5% >=24, 3.9 >26.9	Y	NA	NA	NA

Conc: concentration; Mot: motility; Morp: morphology; NA: no association; Neg: negative association; Pos: positive association; NL: non-linear association; BMI: body mass index; POP: persistent organic pollutant; SD: standard deviation; SEM: standard error of the mean
*controls for abstinence time through restriction (approximately 2-7 days since last ejaculation) rather than statistical adjustment

Table 1.2. Characteristics of studies of the relationship between BMI and semen quality.

A. Sperm concentration

All studies, n=55				
	No association n=33	**Negative association n=20**	**Positive association n=1**	**Non-linear association n=1**
Study population				
Fertility clinic/andrology lab	19 (57.6)	13 (65.0)	0 (0)	0 (0)
General	10 (30.3)	5 (25.0)	1 (100)	1 (100)
Proven fertile	2 (6.1)	0 (0)	0 (0)	0 (0)
Other	2 (6.1)	2 (10.0)	0 (0)	0 (0)
Age				
Mixed	29 (87.9)	20 (100)	1 (100)	0 (0)
Young	4 (12.1)	0 (0)	0 (0)	1 (100)
Study size				
Large (>600)	14 (42.4)	4 (20.0)	1 (100)	1 (100)
Medium (100-600)	15 (45.4)	9 (45.0)	0 (0)	0 (0)
Small (<100)	4 (12.1)	7 (35.0)	0 (0)	0 (0)
BMI ≥25*				
Low (<30%)	4 (12.1)	2 (10.0)	1 (100)	1 (100)
Medium (30-60%)	15 (45.5)	3 (15.0)	0 (0)	0 (0)
High (>60%)	9 (27.3)	10 (50.0)	0 (0)	0 (0)
Controls for age and abstinence (high quality)				
Yes	17 (51.5)	8 (40.0)	1 (100)	1 (100)
No	16 (48.5)	12 (60.0)	0 (0)	0 (0)
Studies with high-quality analyses, n=27				
	No association n=17	**Negative association n=8**	**Positive association n=1**	**Non-linear association n=1**
Study population				
Fertility clinic/andrology lab	8 (47.1)	4 (50.0)	0 (0)	0 (0)
General	7 (41.2)	3 (37.5)	1 (100)	1 (100)
Proven fertile	2 (11.8)	0 (0)	0 (0)	0 (0)
Other	0 (0)	1 (12.5)	0 (0)	0 (0)
Age				
Mixed	14 (82.4)	8 (100)	1 (100)	0 (0)
Young	3 (17.6)	0 (0)	0 (0)	1 (100)
Study size				
Large (>600)	9 (52.9)	3 (37.5)	1 (100)	1 (100)
Medium (100-600)	8 (47.1)	3 (37.5)	0 (0)	0 (0)
Small (<100)	0 (0)	2 (25.0)	0 (0)	0 (0)
BMI ≥25*				
Low (<30%)	3 (17.6)	1 (12.5)	1 (100)	1 (100)
Medium (30-60%)	6 (35.3)	1 (12.5)	0 (0)	0 (0)
High (>60%)	6 (35.3)	5 (62.5)	0 (0)	0 (0)

BMI: body mass index
All data reported as n (percent)
*percentages do not add up to 100 because not all studies reported BMI distribution

B. Motility

All studies, n=50				
	No association n=36	**Negative association n=12**	**Positive association n=1**	**Non-linear association n=1**
Study population				
Fertility clinic/andrology lab	23 (63.9)	7 (58.3)	0 (0)	1 (100)
General	10 (27.8)	3 (25.0)	1 (100)	0 (0)
Proven fertile	2 (5.6)	0 (0)	0 (0)	0 (0)
Other	1 (2.8)	2 (16.7)	0 (0)	0 (0)
Age				
Mixed	32 (88.9)	12 (100)	1 (100)	1 (100)
Young	4 (11.1)	0 (0)	0 (0)	0 (0)
Study size				
Large (>600)	14 (38.9)	4 (33.3)	1 (100)	1 (100)
Medium (100-600)	16 (44.4)	6 (50.0)	0 (0)	0 (0)
Small (<100)	6 (16.7)	2 (16.7)	0 (0)	0 (0)
BMI ≥25*				
Low (<30%)	6 (16.7)	2 (16.7)	0 (0)	
Medium (30-60%)	15 (41.7)	2 (16.7)	1 (100)	
High (>60%)	10 (27.8)	8 (66.7)	0 (0)	
Controls for age and abstinence (high quality)				
Yes	19 (52.8)	6 (50.0)	1 (100)	0 (0)
No	17 (47.2)	6 (50.0)	0 (0)	1 (100)

Studies with high-quality analyses, n=26			
	No association n=19	**Negative association n=6**	**Positive association n=1**
Study population			
Fertility clinic/andrology lab	8 (42.1)	4 (66.7)	0 (0)
General	9 (47.4)	1 (16.7)	1 (100)
Proven fertile	2 (10.5)	0 (0)	0 (0)
Other	0 (0)	1 (16.7)	0 (0)
Age			
Mixed	15 (78.9)	6 (100)	1 (100)
Young	4 (21.1)	0 (0)	0 (0)
Study size			
Large (>600)	10 (52.6)	3 (50.0)	1 (100)
Medium (100-600)	8 (42.1)	2 (33.3)	0 (0)
Small (<100)	1 (5.3)	1 (16.7)	0 (0)
BMI ≥25*			
Low (<30%)	6 (31.6)	0 (0)	0 (0)
Medium (30-60%)	4 (21.1)	2 (33.3)	1 (100)
High (>60%)	6 (31.6)	4 (66.7)	0 (0)

BMI: body mass index
All data reported as n (percent)
*percentages do not add up to 100 because not all studies reported BMI distribution

C. Morphology

All studies, n=41			
	No association n=31	**Negative association n=8**	**Positive association n=2**
Study population			
Fertility clinic/andrology lab	21 (67.7)	2 (25.0)	1 (50.0)
General	6 (19.4)	4 (50.0)	1 (50.0)
Proven fertile	2 (6.4)	0 (0)	0 (0)
Other	2 (6.4)	2 (25.0)	0 (0)
Age			
Mixed	27 (87.1)	8 (100)	2 (100)
Young	4 (12.2)	0 (0)	0 (0)
Study size			
Large (>600)	10 (32.3)	4 (50.0)	1 (50.0)
Medium (100-600)	16 (51.6)	3 (37.5)	1 (50.0)
Small (<100)	5 (16.1)	1 (12.5)	0 (0)
BMI ≥25*			
Low (<30%)	4 (12.2)	2 (25.0)	1 (50.0)
Medium (30-60%)	12 (38.7)	1 (12.5)	0 (0)
High (>60%)	10 (32.3)	5 (62.5)	1 (50.0)
Controls for age and abstinence (high quality)			
Yes	15 (48.4)	5 (62.5)	2 (100)
No	16 (51.6)	3 (37.5)	0 (0)
Studies with high-quality analyses, n=22			
	No association n=15	**Negative association n=5**	**Positive association n=2**
Study population			
Fertility clinic/andrology lab	8 (53.5)	2 (40.0)	1 (50.0)
General	5 (33.3)	2 (40.0)	1 (50.0)
Proven fertile	2 (13.3)	0 (0)	0 (0)
Other	0 (0)	1 (20.0)	0 (0)
Age			
Mixed	11 (73.3)	5 (100)	2 (100)
Young	4 (26.7)	0 (0)	0 (0)
Study size			
Large (>600)	7 (46.7)	3 (60.0)	1 (50.0)
Medium (100-600)	8 (53.3)	1 (20.0)	1 (50.0)
Small (<100)	0 (0)	1 (20.0)	0 (0)
BMI ≥25*			
Low (<30%)	4 (26.7)	1 (20.0)	1 (50.0)
Medium (30-60%)	4 (26.7)	1 (20.0)	0 (0)
High (>60%)	5 (33.3)	3 (60.0)	1 (50.0)

BMI: body mass index
All data reported as n (percent)
*percentages do not add up to 100 because not all studies reported BMI distribution

Figure 1.1. Outline of search strategy.

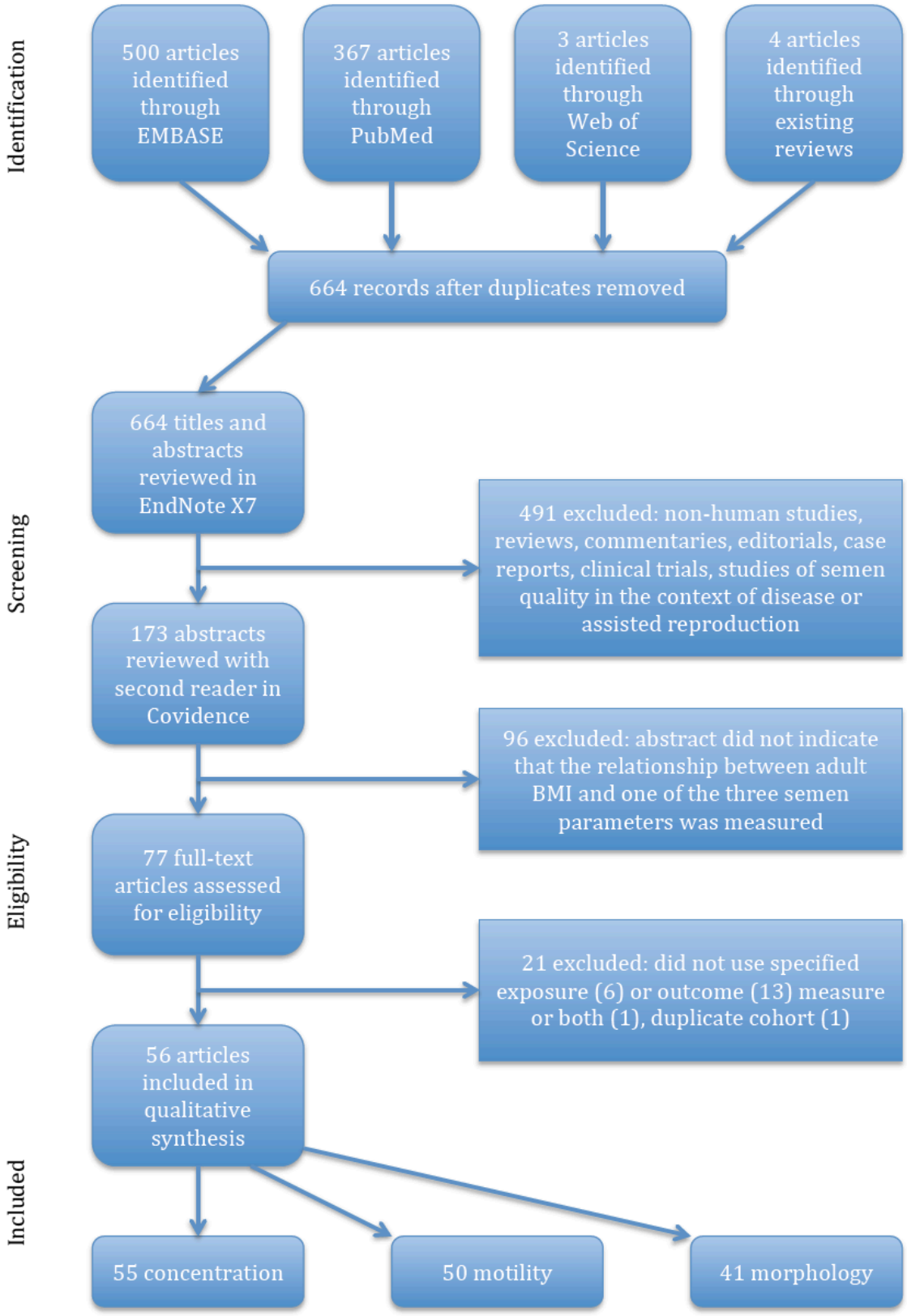
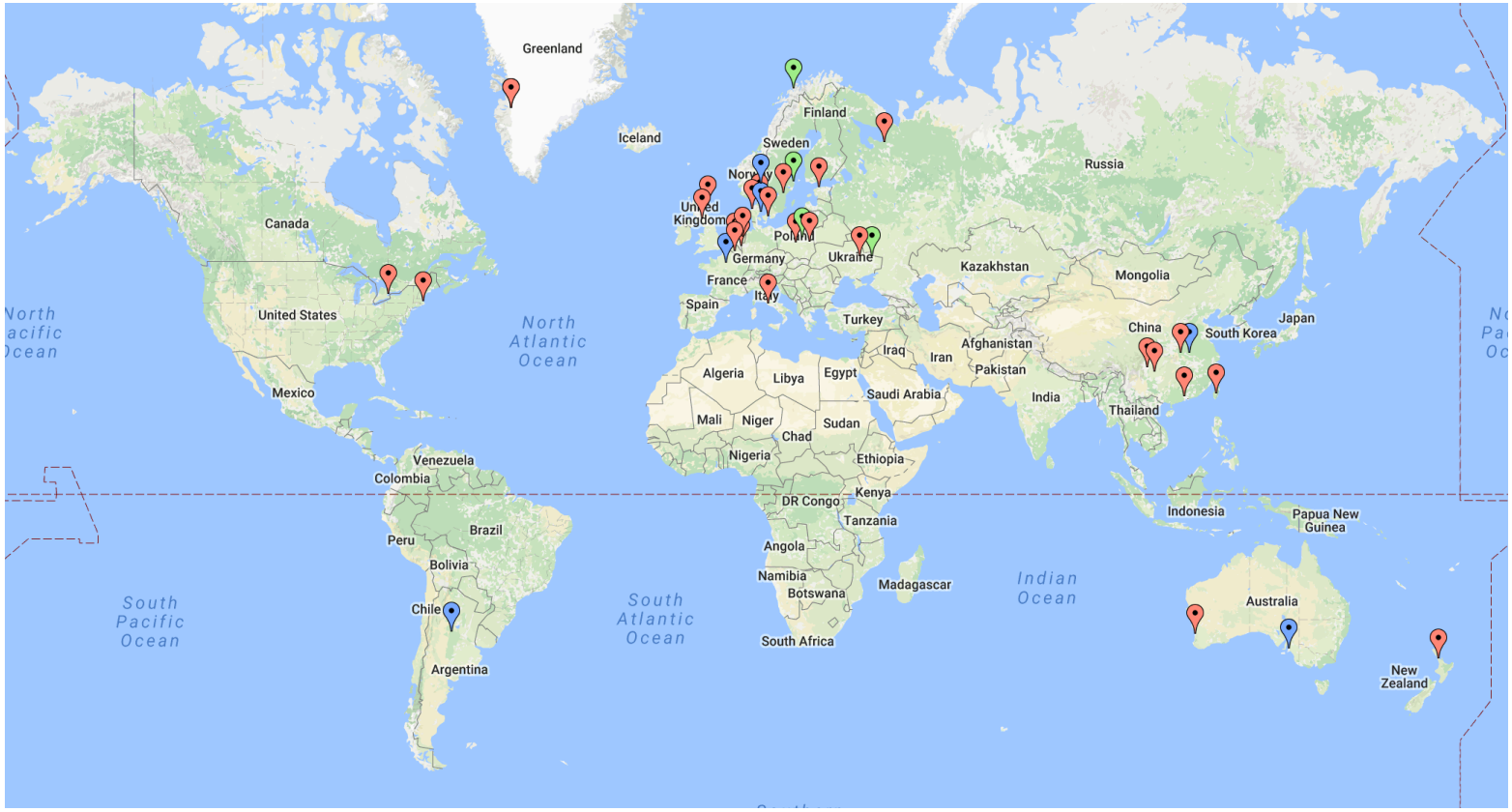


Figure 1.2. Studies with high-quality analyses of BMI and semen quality.

A. Sperm concentration

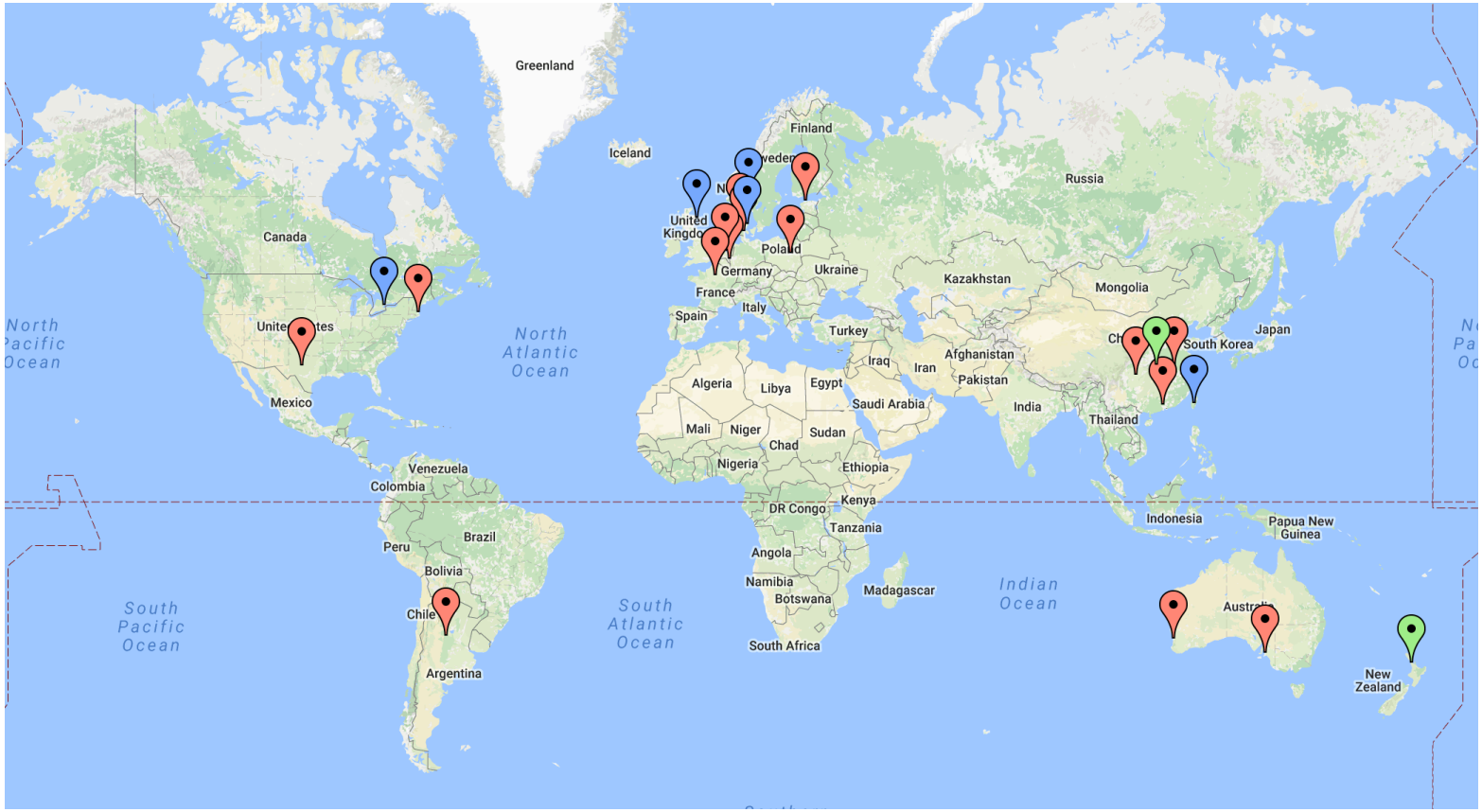
Red: no association
Blue: negative association
Green: positive association

B. Motility



Red: no association
Blue: negative association
Green: positive association

C. Morphology



Red: no association
Blue: negative association
Green: positive association

Supplemental Table 1.1. PRISMA checklist.

Section/topic	#	Checklist item	Page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	6
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	6-7
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	8-11
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	11
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	11-12
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	11
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	11, Sup. Table 1.2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	12
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	12-13
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	N/A
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	N/A
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	13

Supplemental Table 1.2. Search terms and inclusion/exclusion criteria.

EMBASE

('birth weight'/exp OR 'birth weight' OR 'body weight'/exp OR 'body mass'/exp OR 'body mass index' OR 'bmi' OR 'obesity'/exp OR 'obesity' OR 'overweight') AND ('sperm'/exp OR 'spermatozoon'/exp OR 'spermatozoon density'/exp OR 'spermatozoon motility'/exp OR 'sperm quality'/exp OR 'semen analysis'/exp OR 'spermatozoon count'/exp or 'semen quality' or 'semen parameters') AND [english]/lim AND ([article]/lim OR [article in press]/lim) AND [humans]/lim AND [abstracts]/lim

PubMed

((((((((((birth weight[MeSH Terms]) OR body weight[MeSH Terms]) OR body mass index[MeSH Terms]) OR adiposity[MeSH Terms]) OR overweight[MeSH Terms]) OR obesity[MeSH Terms]) OR body mass index[Text Word]) OR bmi[Text Word]) OR birth weight[Text Word]) OR overweight[Text Word]) OR obesity[Text Word]) AND ((((((((semen[MeSH Terms]) OR semen quality[MeSH Terms]) OR semen analysis[MeSH Terms]) OR spermatozoa[MeSH Terms]) OR sperm count[MeSH Terms]) OR sperm motility[MeSH Terms]) OR semen quality[Text Word]) OR semen parameters[Text Word])) AND (Humans[Mesh] AND English[lang])

Inclusion criteria: A statistical test of the relationship between BMI and one or more of three semen parameters: sperm concentration, motility, and morphology

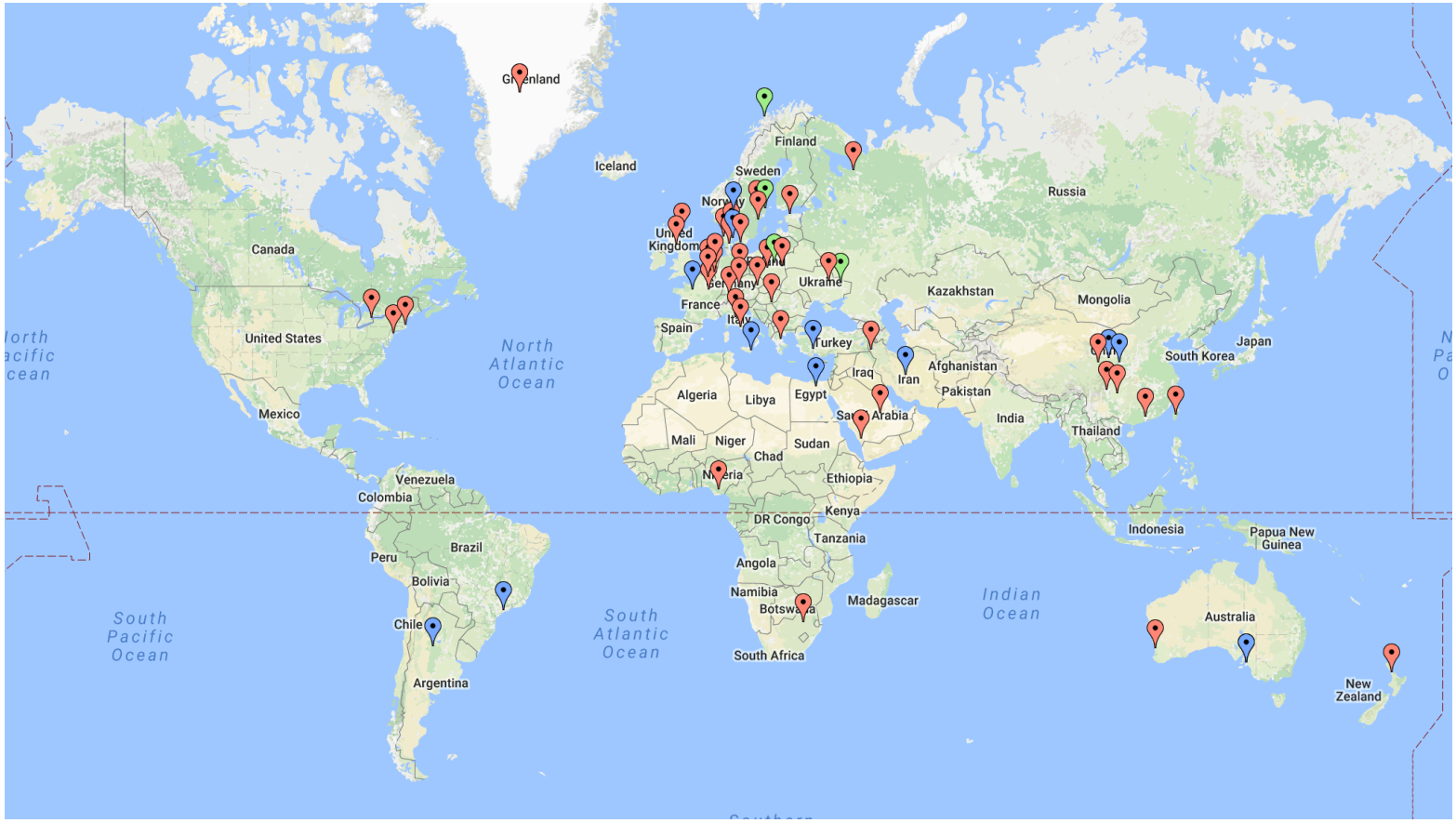
Exclusion criteria: Animal studies, non-empirical articles (e.g., reviews, commentaries/editorials, clinical guidelines), clinical trials, case reports, studies of female infertility and reproduction, and studies of semen quality in the context of research into ART procedures and outcomes, male contraceptives, childhood cancer treatment, genetic/chromosomal abnormalities, hormonal or genitourinary conditions (e.g., varicocele, prostate inflammation, vasectomy reversal, congenital adrenal hyperplasia) or other diseases (e.g., chronic kidney disease, sickle cell disease)

Supplemental Figure 1.1. All studies of BMI and semen quality.

A. Sperm concentration

Red: no association
Blue: negative association
Green: positive association

B. Motility



Red: no association
Blue: negative association
Green: positive association

C. Morphology

Red: no association
Blue: negative association
Green: positive association

CHAPTER 2. THE RELATIONSHIP OF BIRTH WEIGHT AND OF ADIPOSITY ACROSS THE LIFE COURSE TO SEMEN QUALITY IN MIDDLE AGE: RESULTS FROM THE STUDY OF THE ENVIRONMENT AND REPRODUCTION FOLLOW-UP TO THE CHILD HEALTH AND DEVELOPMENT STUDIES

2.1 ABSTRACT

Introduction: Adiposity has been identified as a potentially modifiable risk factor for poor semen quality, yet prior studies of body mass index (BMI) and semen quality have been inconclusive, with most finding no linear association between current BMI and any of the three primary semen quality measures: sperm concentration, percent motile sperm, and percent sperm with normal morphology.

Objectives: Using longitudinal data from the Child Health and Development Studies birth cohort merged with cross-sectional data from the Study of the Environment and Reproduction follow-up, we 1) examined independent relationships of birth weight for gestational age and of adiposity measures throughout early childhood and adulthood with semen quality, 2) tested critical period and cumulative life course models, and 3) explored whether changes in adiposity within childhood or adulthood, or across the lifespan are associated with semen outcomes in middle age.

Methods: One hundred ninety-three non-azoospermatic participants who provided semen samples were included in our analytic sample. In addition to birth weight for gestational age percentile, we created age-specific adiposity measures for three time points in childhood and calculated BMI for three periods in adulthood, then tested whether each individually predicted sperm concentration, percent progressive motility, or percent normal morphology in multivariate regression models. We also ran a series of nested models to test critical period effects, as well as models that used cumulative adiposity scores and trajectory variables as predictors.

Results: We found a statistically significant positive association between birth weight for gestational age percentile and sperm concentration and null or negative associations between childhood adiposity measures and sperm concentration. Adiposity in adulthood, but not childhood, was negatively associated with percent progressive motility, with stronger associations for those who were overweight or obese in

their 20s and a corresponding cumulative effect as they aged. Although not statistically significant, those who were overweight and obese in adulthood had increased odds of low percent normal morphology, with stronger associations for those who were overweight or obese in their 20s.

Conclusion: Our findings of different effects of birth weight, childhood and adult adiposity measures on sperm concentration, motility, and morphology suggest various biological mechanisms by which these aspects of semen quality may be determined. Additional life course studies of adiposity and semen quality are warranted to confirm our results and expand them to include the important developmental periods of puberty and adolescence.

2.2 INTRODUCTION

Adiposity has been identified as a potentially modifiable risk factor for poor semen quality. Numerous studies of body mass index (BMI), a convenient—albeit crude--measure of adiposity, and various semen parameters have been conducted among adult men in diverse study populations around the world. With a single exception(126), all of them have been cross-sectional, allowing only for inference of the effect of current adiposity on sperm production. While these analyses may capture the effect of BMI on the 74-day process of spermatogenesis (from the mitotic division of the spermatogonia (stem cells of the male germinal cell line) through their daughter spermatocytes' proliferation, migration, meiotic division, maturation, and differentiation into spermatozoa(41)), this represents only the culmination of a developmental trajectory that begins during fetal life and continues through puberty, adolescence, and into adulthood. There remains a wide gap in our understanding of how birth weight, as well as adiposity in infancy, childhood, young adulthood, and middle age may individually and cumulatively influence the biological mechanisms that determine different aspects of semen quality.

The results of prior studies of BMI and semen quality have been inconclusive, with most finding no linear association between BMI and the three common semen quality measures: sperm concentration (millions/mL), percent motile sperm, and percent sperm with normal morphology (reviewed in (38-40)). There is some indication that there may be a nonlinear relationship between BMI and low sperm concentration, as a meta-analysis of 21 studies by Sermondade et al. found that underweight and overweight/obese men were at higher risk of low sperm count and concentration compared to men in the normal BMI range(39). A more recent meta-analysis by Campbell et al. found no relationship between BMI and sperm concentration, but suggests that any negative relationship between BMI and motility or morphology may be driven by men in the obese category(40). The overall inconsistency of these studies' results likely stems from differences among their study samples in terms of racial/ethnic composition, environmental exposures, age (most included men of mixed ages, ranging from adolescence through old age, while a few focused exclusively on young men), sample BMI distribution, and fertility status (most recruited from fertility clinics, yielding a preponderance of subfertile men, while some recruited only fertile men and others recruited from the general population).

Fetal growth, for which birth weight for gestational age is commonly used as a proxy(127), provides a convenient, if imprecise, marker of conditions of the intrauterine environment that may exert a lasting influence on male reproductive potential. We found three published studies that examined the relationship between birth weight and semen quality. As a follow-up study of 347 men whose mothers were part of the Danish Healthy Habits for Two pregnancy cohort, Ramlau-Hansen et al. found no association between birth weight and semen quality at age 18-21 years(126). Olsen et al. reported reduced sperm concentration among men who were born between 3,000 and 3,999 g compared to those with lower or higher birth weights(128). Auger et al. found higher birth weight to be associated with an increase in abnormally shaped sperm(129).

Additionally, there may be critical periods in childhood when adiposity may indelibly affect later-life semen quality, possibly through alterations in pubertal timing and development. While numerous studies have documented an association between increased BMI in childhood and early onset of puberty in girls, research on boys has been sparse. Nevertheless, a few studies in varied populations have suggested an inverse relationship between childhood BMI and age at puberty in boys(130-135). Both early (0-12 months) and middle (2-8 years) childhood have been identified as potentially critical periods during which increased adiposity may affect male pubertal development(134, 135). While the biological mechanisms underlying these observed associations are not clear, it is theoretically possible that whatever hormonal pathways implicated in the timing of male puberty that may be disrupted by early childhood adiposity may also affect the maturation and proliferation of Sertoli and/or Leydig cells in the testes, thereby influencing semen quality in adulthood. In the one study we found that explored childhood adiposity and adult semen quality, Ramlau-Hansen et al. found no association between prepubertal BMI (age 5-8 years) and semen quality in young adulthood(126). It is also possible that the risk of poor semen quality accumulates with exposure to increased adiposity over the life course.

The current study, conducted among participants in the Study of the Environment and Reproduction (SER) follow-up to the Child Health and Development Studies (CHDS) birth cohort, provided a unique opportunity to explore the relationship of birth weight and of adiposity across the life course to semen quality in middle age. Drawing on the methodological framework outlined by Elwer et al.(136), we conducted analyses that tested the two conceptual models described by Ben-Shlomo and

Kuh in their seminal paper on life course epidemiology(42): critical period, in which an exposure at a particular point in biological development results in a permanent change in physiologic function that has lifelong effects, and accumulation of risk, in which the effect of an exposure increases according to the number of times the individual was exposed. In a secondary analysis, we also explored how changes in adiposity across childhood, adulthood, and the entire lifespan might affect semen quality in middle age.

2.3 METHODS

2.3.1 Study population

The current analysis includes 193 middle-age men (mean age 43) whose mothers participated in the CHDS during pregnancy. The CHDS is a birth cohort that enrolled 98% of eligible pregnant women who were members of the Kaiser Foundation Health Plan in and around Oakland, California, between 1959 and 1966. Women were recruited early to mid-pregnancy, at which time they provided a blood sample and underwent an extensive face-to-face interview that collected demographic, lifestyle, and background medical information about both them and their partners, as well as the women's reproductive history and details of their current pregnancy. Follow-up data included additional prenatal blood samples, cord blood samples, questionnaires, and child growth and medical information, which were collected at regular intervals through age five. A subset of children was followed through puberty and adolescence.

SER was one of a series of follow-up studies of CHDS child participants designed to investigate the impact of prenatal exposures and childhood growth and development on health in middle age. Men were recruited between December, 2005 and April, 2008 (mean age 43 years) with the aim of examining the effect of prenatal exposure to environmental chemical exposures on reproductive health, specifically time-to-pregnancy and semen quality. Men were eligible to participate if they had data on birth length and weight, at least one same-day height and weight measure between age 6 months and 5.5 years, maternal prenatal interview data, and an adequate volume of sera obtained from the mother in mid-pregnancy to measure maternal thyroid hormone and in the immediate postpartum period to assess the presence of environmental chemicals. They also needed to live within 100 miles of the Kaiser Oakland Clinic, as the study entailed two clinic visits. Exclusion criteria included gestational age <37 weeks and major

congenital abnormalities. Attempts were made to contact 1,202 of the 3,531 ostensibly eligible participants from the recruitment pool. Of the 654 (54%) who were successfully reached, 568 met eligibility criteria, and 338 (60%) of that group consented to participate and provided interviews. Of those, 196 (58%) additionally provided at least one semen sample (Figure 2.1)(137). SER participants who provided semen samples were comparable to the underlying non-preterm CHDS cohort in terms of gestational age and birth weight, but were less likely to be white. They were also less likely to be white and had lower gestational age compared to SER participants who did not provide semen samples (Supplemental Table 2.1). The current analyses exclude 3 SER participants who were azoospermatic (had no sperm cells in their semen).

2.3.2 Study protocol

Upon recruitment, participants traveled to the Kaiser Oakland Clinic, where they underwent a one-hour in-person interview; were weighed, measured, and had blood pressure taken; and provided semen and blood samples. Topics covered in the interview included demographic information, health and medical history, reproductive health history, psychosocial stressors, lifestyle characteristics, and deleterious exposures. Blood samples were drawn following semen collection. Participants returned to the clinic approximately 2 weeks later to provide a second semen sample. Details of the study protocol have been published previously(137).

2.3.3 Exposure measures

2.3.3.1 Birth weight for gestational age

We created sex-specific birth weight for gestational age percentiles (bw/ga) for participants using continuous curves derived from United States Natality datasets containing information on male singleton infants born to United States resident mothers in 1999-2000(138). Although these curves were created more than three decades after the last of the CHDS children were born, they are the first continuous curves available based on the entire US population; prior curves only provided data for specific percentiles (e.g., 10^{th}, 50^{th}, 90^{th})(139-142) and were sometimes based on exclusively white

populations(139, 140). Even if the bw/ga curve did shift in the intervening years, the rank order of the participants' percentiles would remain the same.

2.3.3.2 Adiposity measures

Because measures of adiposity are not standard across the life course, we created several different variables appropriate to the critical periods hypothesized to be potentially relevant to semen quality. For the first two critical periods, at approximately 4 and 12 months of age, we calculated sex-specific weight-for-age percentiles (wt/age) and sex-specific weight-for-height percentiles (wt/ht) using a SAS program provided by the Centers for Disease Control and Prevention (CDC) that draws on data from the 2000 CDC weight-for age and height-for-age growth charts for birth through 36 months(143). Although the first CDC growth charts became available in 1977, the 0-36 month curves were based on data from the longitudinal growth study of the Fels Research Institute in Yellow Springs, Ohio, which included mainly formula-fed white middle-class infants. The 2000 charts are based on data from the National Health and Nutrition Examination Survey (NHANES) I (1971-74), NHANES II (1976-1980), NHANES III (1988-94), and national birth certificate data from United States Vital Statistics that better reflect the racial/ethnic diversity of the CHDS cohort(144). For the third critical period, at approximately 4 years of age, we used the CDC SAS program to calculate sex-specific BMI-for-age percentiles (ssBMI) based on 2000 CDC BMI-for-age growth charts**.** These charts reflect data from the three NHANES waves mentioned above as well as two earlier surveys, the National Health Examination Survey (NHES) II (1963-5) and NHES III (1966-70)(144). Categorical adiposity variables were created from the continuous variables using CDC cut points for child under/normal weight (<85th percentile), overweight (85th to <90th percentile), and obesity (≥95th percentile).

Continuous BMI measures were calculated for three time periods in adulthood. Measured weight and height at the time of semen collection yielded current BMI. BMI in participants' 20s and 30s was calculated from recalled weight, under the assumption that height is stable between early adulthood and middle age. Categorical adult BMI measures were created using World Health Organization (WHO) cut points for under/normal weight (<25 kg/m^2), overweight (25 to <30 kg/m^2), obese (30 to <35 kg/m^2), and morbidly obese (≥35 kg/m^2).

Cumulative overweight and cumulative obesity scores were created by allocating 1 point for every time period in which a subject was classified as either overweight/obese (≥85th percentile for child measures, ≥25 kg/m^2 for adult measures) or simply obese (≥95th percentile for child measures, ≥30 kg/m^2 for adult measures). In addition to a 0-6 point scale that covered all 6 ages at which adiposity was measured, we created separate 0-3 point scales for cumulative child and adult overweight/obesity and obesity. Finally, we generated nominal categorical variables that represented trajectories of adiposity from 4 months to 4 years of age and from participants' 20s to their age at the time of the study, as well as two four-category lifetime trajectory variables representing ever/never overweight/obese and ever/never obese at any point in childhood vs. adulthood (Supplemental Table 2.2).

2.3.4 Outcome measures

Participants were invited to provide two semen samples approximately two weeks apart, each after 2-5 days of ejaculatory abstinence. Only complete samples were analyzed; incomplete samples were discarded and men were asked to return at a later date. Semen analysis was performed according to a standard protocol: volume, sperm concentration, and percent motile sperm were assessed at the Kaiser Clinic within one hour and seminal smears were prepared and sent to the Andrology Laboratory at the University of California, Davis, where they were stained in order to determine percent normal morphology, which was classified according to strict criteria(145). Details of the semen analysis(137) and quality controls used throughout the procedure(146) have been described previously. Because the intraclass correlation coefficients for the three semen quality measures among men who provided two samples indicated good reliability between measures (ICC_{conc} = 0.70, ICC_{mot} = 0.65, ICC_{morph} = 0.80)(137), the mean value of each parameter was used when two were available and single values were used when only one was available.

Semen parameters were modeled both as continuous variables and dichotomously using 2010 WHO reference values for subfertility: sperm concentration <15 million/mL, percent progressive motility <32%, percent normal morphology (strict criteria) <4%(147). These values were updated based on samples from more than 1953 men in 8 countries who had known time to pregnancy ≤12 months(147), the commonly accepted definition of couple fertility(148). There has been some criticism of the

methodology behind the 2010 WHO reference values(149) and debate as to whether cutoff values for what is considered "normal" (the 5th centile of the reference ranges) are warranted when there is no evidence of a biological threshold effect(5). Nevertheless, these reference values are frequently cited in the literature and clinical practice as indicators of male subfertility. We additionally created a 3-level combined outcome measure for subfertility that categorized participants according to the number of parameters they had below the 2010 WHO reference values (0, 1, 2-3 parameters).

2.3.5 Covariates

Information on behavioral, sociodemographic, medical, and psychosocial risk factors for adiposity at different life stages and poor semen quality was assessed via questionnaire administered by trained interviewers. Prenatal variables were available, but were hypothesized to be linked to the outcomes primarily through their contribution to the adiposity exposures; we therefore chose not to include them in the analyses in order not to incur bias from overadjustment(150). Covariates that were identified as potential confounders and predictors of the outcomes based on a review of the literature and causal diagrams (Supplemental Figure 2.1) included: participants' abstinence time (continuous, minutes), current age (continuous, years), race (white, black, other), education (high school, some college, bachelor's degree, graduate level), annual income (<$50,000, $50-100,000, $100-150,000, >$150,000), smoking status (current vs. not current), alcohol consumption (continuous, average g/week during past 12 months), caffeine consumption (continuous, average mg/week during past three months), exercise (continuous, average minutes/week during past 3 months), ever diagnosed with a sexually transmitted infection (Y/N), current hormone use (Y/N), and current employment status (employed/unemployed). Current stress and depression variables were also considered, as prior analyses in this data set found perceived stress, stressful life events, and unemployment to be associated with our semen outcomes(151). Available stress measures included the validated abbreviated 10-item Perceived Stress Scale (PSS)(152); a shortened form of the Life Events Inventory (LEI)(153), including the top 10 stressors of men from an occupational sample(154); the 16-item Job Content Questionnaire (JCQ)(155); and the 20-item Center for Epidemiologic Studies Depression scale (CES-D)(156). PSS and CES-D were modeled continuously; LEI was modeled categorically (0, 1, 2 or more stressful life events). Those above

the sample median job demand score and below the sample median job control score on the JCQ were categorized as high strain; the rest were categorized as low strain. A three-level variable was created in which participants were categorized as unemployed, employed with low job strain, or employed with high job strain.

2.3.6 Statistical analyses

Univariate analyses were performed to describe the study sample and to compare it to both the source population of male CHDS participants and to SER participants who did not provide semen samples. We also examined histograms of the three semen parameters. In bivariate analyses, analysis of variance was used to compare means of continuous exposure and outcome variables (adiposity measures and the three semen parameters) according to levels of categorical covariates. Nonparametric Spearman correlations were used to assess associations between continuous variables. Chi-square tests were used to detect bivariate associations between categorical covariates. The relationships of adiposity in participants' 20s, 30s, and at the time of the SER study to their semen quality were explored through data visualization techniques including scatterplots with loess smoothing curves and bar graphs showing the frequencies of each of the three dichotomous variables representing poor semen quality according to the WHO reference levels by BMI category.

In regression analyses, we decided *a priori* to include participants' race, current age, and abstinence time in all final models. To determine which additional covariates warranted inclusion in the final models, separate simple linear regression models were run to examine the association between bw/ga and each adiposity measure and the three outcome variables. Hypothesized confounders and predictors of the outcomes that changed the estimated regression coefficient of the predictor in the simple model by at least 50% of the standard error or were associated with the outcome with a p-value <0.10 were included in the relevant covariate-adjusted models. Bw/ga, reflecting the cumulative effect of the prenatal environment on fetal growth, was considered as a primary exposure, but was also included as a covariate in all adiposity models as a proxy measure of the intrauterine environment. When the wt/ht variable was used to capture adiposity at 4 and 12 months, models were additionally adjusted for the participants' age when weight and height were taken, measured in days.

To test whether bw/ga and each of the adiposity measures was independently associated with each of the three semen outcomes, we ran a series of three models: 1) including only the main effect, 2) including the main effect, current age, race, and abstinence time, and 3) including the main effect, current age, race, abstinence time, and the additional covariates relevant to each parameter (bw/ga was additionally included in all childhood and adult adiposity models). Using linear regression for continuous outcomes and logistic regression for binary outcomes, this series was run with 1) continuous predictors and outcomes, 2) continuous predictors and dichotomous outcomes (based on WHO reference values), 3) categorical predictors (based on cut points for overweight and obesity) and continuous outcomes, and 4) categorical predictors and dichotomous outcomes. The same strategy was applied to the multinomial logistic models for the 3-level poor semen quality outcome variable based on the WHO reference levels to assess its association with either continuous or categorical predictors. In this case, dichotomous predictor variables were used (overweight/obese vs. normal and obese vs. normal/overweight) because the models failed to converge when the three-level categorical predictors were used.

To test whether any of the adiposity measures exerted a critical period effect, a series of covariate unadjusted and adjusted nested linear regression models was run for each semen outcome. First, each of the three outcome variables was regressed on 4-month wt/age controlling for bw/ga, with and without covariates. Subsequent continuous critical period measures were added sequentially, culminating with current BMI. The exercise was repeated using 4- and 12-month wt/ht measures. Because the three continuous adult BMI measures were highly correlated and the estimated regression coefficients could be in opposite directions yielding ambiguous interpretations(157), in alternative analyses the series was run using variables that represented the differences in percentiles or BMI scores between adjacent time periods.

The cumulative effect of adiposity was assessed by regressing the continuous semen outcomes on 1) the cumulative overweight/obese variable that included all 6 time points, 2) the cumulative overweight/obese variable that included the 3 childhood time points, and 3) the cumulative overweight/obese variable that included the 3 adult time points. We repeated this series using the three cumulative obesity variables, and then repeated the entire process using the dichotomous semen outcome variables.

Finally, to test whether participants' adiposity trajectory between 4 months and 4 years of age, between 20s and middle age, and between childhood and adulthood were related to semen quality, we regressed both continuous and dichotomous semen outcome measures on the nominal categorical childhood, adulthood, and lifespan trajectory variables with and without covariate adjustment. All analyses were performed in SAS 9.3 (SAS Institute, Cary, NC).

2.4 RESULTS

2.4.1 Descriptive analyses

The 193 SER participants included in these analyses had a mean sperm concentration of 73.6 million sperm/mL (range: 2.1-429.9 million/mL), mean percent progressive motility of 40.0% (range: 0-76.5%), mean percent normal morphology of 7.6% (range: 0-20.0%), and mean current BMI of 28.8 kg/m^2 (range: 19.4-44.3 kg/m^2). Participants did not differ significantly on demographic or behavioral characteristics according to their BMI category (Table 2.1). 9.8% of the sample was below the WHO reference level for sperm concentration (<15 million/mL), 32.6% was below the reference level for percent progressive motility (<32%), and 22.4% was below the reference level for percent normal morphology (<4%). 58.6% of the sample met none of the WHO criteria for poor semen quality, while 6.3% met all three (Supplemental Figure 2.2). Whereas motility and morphology were approximately normally distributed, sperm concentration was found to be right-skewed and was square-root transformed (Supplemental Figure 2.3).

In bivariate analyses of potential covariates and semen parameters, African-American men had significantly lower percent progressive motility (32.3% vs. 41.3% and 43.3%) and normal morphology (6.6% vs. 7.3% and 9.5%) compared to white men and men of other racial backgrounds. Men who earned ≤$50,000 per year had significantly lower percent progressive motility compared to the three higher income groups (35.5% vs. 41.6%, 45.0%, 41.3%, respectively). Similarly, men in the lowest education group (≤high school) tended to have lower percent progressive motility compared to the three higher education groups (34.2% vs. 41.3%, 39.4%, 42.3%, respectively), although this difference did not reach statistical significance. In chi-square tests, income and education were each strongly associated with race

in this sample (p-value < 0.0001). Current smokers had significantly lower percent progressive motility (30.8% vs. 42.0%) compared to nonsmokers. Compared to employed men, unemployed men had significantly lower percent progressive motility (29.2% vs. 41.7%) and percent normal morphology (5.9% vs. 7.9%). Those who had experienced 2 or more adverse life events in the preceding 12 months also had significantly lower percent progressive motility and normal morphology compared to those who experienced fewer than 2. Age was significantly negatively correlated with all three semen outcomes, while perceived stress was negatively correlated with motility and morphology and depression was negatively correlated with motility. Increased abstinence time was significantly positively correlated with increased sperm concentration (Supplemental Table 2.3).

The three semen parameters were all positively correlated with one another (range of coefficients: 0.37-0.52) (Supplemental Table 2.4). The three childhood adiposity measures were also positively correlated (range of coefficients: 0.35-0.67), as were the three adult adiposity measures (range of coefficients: 0.69-0.84). The only childhood adiposity measure to be associated with adult BMI was 4-year-old ssBMI, which was significantly positively correlated with all of the adult measures (range of coefficients: 0.19-0.23) (Supplemental Table 2.5).

2.4.2 Independent associations

2.4.2.1 Concentration

Bw/ga was positively associated with sperm concentration. For every percentile increase in bw/ga, square-root sperm concentration increased by 0.02×10^3 sperm/mL in covariate-adjusted analyses (95% confidence interval (CI) [0.00, 0.04]). Continuous childhood adiposity measures were not associated with sperm concentration, but categorical measures tended to be increasingly negative as time since birth elapsed. In particular, overweight at 4 years was significantly negatively associated with sperm concentration (b_{adj} = -1.70; 95% CI [-3.19, -0.21]). In logistic models, neither bw/ga nor any of the adiposity measures was associated with low sperm concentration based on the WHO reference level (Table 2.2a).

2.4.2.2 Motility

In contrast to the results for concentration, neither bw/ga nor early childhood adiposity measures was significantly associated with percent progressive motility. Participants who were obese in their 20s or 30s tended to have lower percent progressive motility compared to men with BMI <25 kg/m^2 in linear models (b_{adj} = -8.08; 95% CI [-17.13, 0.97], p-value = 0.08 and b_{adj} = -5.98; 95% CI [-12.46, 0.49], p-value = 0.07,respectively). Using the dichotomous outcome variable based on the WHO reference level for low percent progressive motility, we found that participants with higher BMI in their 20s and 30s had increased odds of low percent progressive motility in covariate-adjusted analyses (OR_{adj} = 1.12, 95%CI [0.996, 1.25], p-value = 0.06 and OR_{adj} = 1.09, 95% CI [0.998, 1.19], p-value = 0.06, respectively). Participants who were overweight in their 20s had 2.58 times the covariate-adjusted odds of low motility compared to those with BMI <25 kg/m^2 (95% CI [1.10, 6.01]) and participants who were obese in their 30s had 3.18 times the covariate-adjusted odds compared to those with BMI <25 kg/m^2 (95% CI [1.05, 9.66]) (Table 2.2b).

2.4.2.3 Morphology

None of the childhood or adulthood adiposity measures was significantly associated with percent normal morphology in covariate-adjusted linear models. Using the WHO-based dichotomous morphology outcome variable and logistic regression, we found that obesity at the time of semen collection was positively associated with low percent normal morphology (OR_{adj} = 1.91; 95% CI [0.70, 5.27], p-value = 0.07) (Table 2.2c).

Results of the analyses of independent associations were similar when wt/ht measures were used instead of wt/age at 4 and 12 months, adjusting for participants' age in days at those examinations.

Loess smoothing curves of semen parameters by adult BMI reflected the findings of our regression analyses. In particular, they made visible the decline in sperm motility associated with overweight in participants' 20s and obesity in their 30s. It was also apparent that there was no association between current BMI and motility (Supplemental Figure 2.4). In bar graphs showing the percent of participants below each semen parameter's WHO reference value by BMI category, increasing BMI category was associated with increasing percentage of participants with low motility at all adult time

points. The relationship attained statistical significance for BMI in participants' 20s (two-sided exact Cochran-Armitage trend test p-value = 0.02) (Supplemental Figure 2.5).

Subfertility, defined as being below one or more of the WHO reference values, was related to adiposity in early adulthood, but not childhood or at the time of the study. Participants with BMI ≥25 kg/m^2 in their 20s had 3.52 times the covariate-adjusted odds of meeting 2-3 WHO subfertility criteria compared to participants who with BMI <25 kg/m^2 (95% CI [1.42, 8.75]), and participants with BMI ≥30 kg/m^2 at the time of semen collection had 2.42 times the covariate-adjusted odds of meeting 2-3 WHO subfertility criteria compared to participants who with BMI <30 kg/m^2 (95% CI [1.04, 5.69]). For each 1-unit increase in BMI in their 30s, participants had on average a 11% increased covariate-adjusted odds of meeting 2-3 subfertility criteria (95% CI [1%, 21%]) (Supplemental Table 2.6).

2.4.3 Critical period models

A critical period effect was identified if adiposity at a particular time point was associated with a semen outcome and maintained that association despite the addition of subsequent adiposity measures to the model(136). None of the child or adult adiposity measures demonstrated a statistically significant critical period effect on any of the semen outcomes (Tables 2.3a-c). The relationship between wt/ht at 12 months and sperm concentration approached statistical significance and remained constant despite the addition of subsequent adiposity measures (b_{adj} = 0.03, 95 % CI [-0.00, 0.05]) (Table 2.3a). Results were comparable when we used categorical adult BMI measures and difference measures, and when we restricted analyses to only those who had adiposity measures at all six time points.

2.4.4 Cumulative models

The results of our analysis of a cumulative effect on semen quality paralleled our findings regarding independent associations. We found no association with sperm concentration when all 6 time points were represented in the cumulative overweight/obese and obesity predictor variables. When we modeled separate 0-3 point scales representing cumulative adiposity in childhood and adulthood, we found negative but not statistically significant associations of increasing cumulative overweight and

obesity with sperm concentration using the childhood scales and no associations using the adulthood scales (Table 2.4a). By contrast, we found no associations between the total and childhood scales and progressive motility, but found a significant negative association using the adult cumulative obesity scale (b_{adj} = -2.60; 95% CI [-5.09, -0.12]) and a corresponding positive association between the adult cumulative overweight/obesity scale and low percent progressive motility (OR_{adj} = 1.33; 95% CI [0.96, 1.86], p-value = 0.09) (Table 2.4b). Both adult cumulative overweight/obesity and obesity scales were positively associated with low percent normal morphology (OR_{adj} = 1.34; 95% CI [0.95, 1.89], p-value = 0.096 and OR_{adj} = 1.48; 95% CI [0.97, 2.26], p-value = 0.07, respectively) (Table 2.4c). When we restricted the adult analyses to only the participants with childhood measures, the associations were attenuated but in the same direction.

2.4.5 Trajectory analyses

In secondary analyses, we examined trajectories of adiposity over the course of childhood (between 4 months and 4 years) and adulthood (between 20s and age at semen collection), as well as over the entire lifespan. We did not find any associations with sperm concentration or percent progressive motility (Tables 2.5a-b). Compared to those who were never obese, those who were normal or overweight in childhood and obese in adulthood had increased percent normal morphology (b_{adj} = 3.52, 95% CI [1.68, 5.36] (Table 2.5c).

2.5 DISCUSSION

In keeping with the majority of published studies, we found no association between current BMI and sperm concentration. Our findings of a positive association between bw/ga and sperm concentration and increasingly negative associations between childhood adiposity measures and sperm concentration suggest that prenatal and early childhood periods may be important developmental windows for testicular Sertoli and Leydig cells, which control spermatogenesis. Sertoli cells, which are the first cells to differentiate in the fetal gonad, play an essential role in fetal and neonatal testicular development, including the subsequent differentiation of Leydig cells that produce the testosterone necessary for

masculinization of the fetus. In puberty, their role shifts to supporting the differentiation, meiosis, and transformation of spermatocytes into spermatozoa, under the hormonal regulation of testosterone produced by nearby Leydig cells. Sertoli cells proliferate during both of these periods; following puberty, they reach functional maturity and no longer multiply(158). Because each Sertoli cell can only support a fixed number of spermatocytes at a time(159, 160), prenatal conditions that affect Sertoli cell proliferation will affect sperm concentration later in life. A study of pregnant ewes fed high and low metabolizable energy diets found those fed the high-energy diets gave birth to heavier lambs and to lambs with significantly higher number of Sertoli cells per testis(161), suggesting that overall fetal growth and Sertoli cell proliferation may respond to common exposures in the intrauterine environment.

Whereas Sertoli cells have two developmental phases, Leydig cells pass through three phases of maturation, from fetal to neonatal to adult Leydig cells, reflected in peaks of testosterone production at 14-18 gestational weeks, 2-3 months of age, and from puberty onward. Postnatally, as Sertoli cells are entering a nonproliferative quiescent phase, Leydig cells are entering their second stage of development. After this second peak, a portion of neonatal Leydig cells regresses into immature Leydig cells, which eventually give rise to adult Leydig cells in puberty(162). Environmental circumstances that interfere with or promote successful regression may eventually influence the number of adult Leydig cells and consequently affect testosterone production in adulthood. Although our finding of a trend toward a critical period effect of adiposity at 12 months on sperm concentration should be interpreted with caution, as the models were unstable due to the collinearity of the childhood measures, they are in line with this proposed biological mechanism.

In contrast to sperm concentration, which was solely influenced by prenatal and early childhood adiposity in our study, progressive motility was associated with adult adiposity measures, and most strongly with young adult measures. Obesity is associated with systemic buildup of excessive reactive oxygen species (ROS) (163)—unstable oxygen-containing molecules generated as a byproduct of cellular respiration--which can adversely affect sperm motility through two possible pathways. First, ROS peroxidize the polyunsaturated fatty acids in the sperm plasma membrane, leading to loss of membrane fluidity and integrity and, consequently, reduced motility(164). Second, excess ROS can damage mitochondrial DNA, reducing ATP production in the mitochondrial membrane(165). Decreased

mitochondrial membrane potential has been associated with reduced sperm motility(166), as ATP is required to power and sustain flagellation(167). Rather than having a critical period effect on motility, the association between overweight and obesity in participants' 20s with reduced motility is likely due to the cumulative effect of obesity and resulting oxidative stress. Because most men gained weight between their 20s and 40s or maintained a BMI ≥25 kg/m^2 (out of 184 who had BMI measures at all three time points, only 41 retained a normal BMI throughout adulthood and 5 lost weight), those who were obese in their 20s were likely to be exposed to excess ROS for the longest period of time prior to semen collection. We noted a slightly weaker association between obesity and motility for BMI in participants' 30s and the weakest association for current BMI.

Our results for morphology were less clear, although as with motility, it appeared to be linked more closely to adult BMI than adiposity in childhood. This may be a reflection of the close relationship between motility and morphology: defects in sperm head, neck, and tail all have the potential to adversely affect sperm motility(168). Sperm heads are shaped in a complex interaction between the developing spermatid and the "nurse" Sertoli cell. First, the round spermatid cell nestles into a specialized region of the Sertoli cell's plasma membrane. Then an elongated scaffold of microtubules and F-actin hoops anchored to the endoplasmic reticulum of the Sertoli cell is formed around the spermatid and squeezes it into the characteristic oval sperm-head shape. Meanwhile, the Golgi apparatus in the Sertoli cell produces two types of vesicles: 1) acrosomal vesicles, which are transported via scaffold microtubules to the top of the spermatid nucleus, where they fuse to form the acrosome, and 2) non-acrosomal vesicles, which are transported to the opposite end of the spermatid, the location of the centrosome, where they contribute to tail development(115). While details of the spermatid head-shaping mechanism are still not completely understood, it is possible that the dysregulation of reproductive hormones associated with obesity, known as hyperestrogenic hypogonadotropic hypoandrogenemia(113), could interfere with its regulation. Furthermore, because of the important role that the Sertoli cell membrane plays in the shaping process, any obesity-induced increase in ROS that weakens the membrane's lipid structure could also prove detrimental to normal sperm morphology.

2.5.1 Strengths

This study has several strengths. Because of the unique nature of the SER follow-up to the CHDS, it is the only study that we know of that has ever been able to explore the relationship of adiposity over the life course to semen quality in middle age. Having this rich resource allowed us to consider multiple life course models and discern between early life and adult influences on all three common semen quality parameters. Because of the in-depth interview of SER participants, we were able to adjust for a range of covariates, including demographic, behavioral, and psychosocial factors. Outcome misclassification was minimized, as semen collection and analysis followed a validated protocol and was carried out in the same laboratory that participated in the National Institute of Child Health and Human Development-funded National Cooperative Reproductive Medicine Network (Fertile Male Study)(169). Mean concentration and motility values in our sample are comparable to several other studies conducted among men drawn from the general population(54, 84, 100), enhancing generalizability. (We were unable to find comparable studies that reported mean percent morphology according to strict criteria.) Additionally, our results are generalizable back to full-term participants in the original CHDS, a diverse population-based cohort.

2.5.2 Limitations

Many of our findings were of borderline statistical significance, reflecting our limited sample size, which was restricted due to the expense involved in tracing participants who had not been contacted for more than 30 years and the challenge of recruiting men willing to provide semen samples. We also lacked childhood adiposity measures beyond 4 years of age; ideally we would have had additional data points in puberty and adolescence that would have allowed us to explore these potentially critical periods and provide a more continuous picture of adiposity across the life course. Because of the overall increase in child adiposity between 1970 and 2000 in the US(170), our use of the 2000 CDC growth charts was likely a source of nondifferential exposure misclassification, which would bias our results toward the null. Reliance on recalled weight during participants' 20s and 30s could also be a source of exposure misclassification, but studies of recalled weight among middle-age men in the United States(171) and United Kingdom(172) found that recalled weight was highly correlated with measured past weight; any

resulting bias would therefore be minimal and toward the null.

2.5.3 Conclusion

Our findings add nuance and complexity to the debate over the relationship of adiposity to semen quality. We found a statistically significant positive association between bw/ga and sperm concentration and increasingly negative associations between childhood adiposity measures and sperm concentration, especially among those in the overweight and obese categories. Adiposity in adulthood, but not childhood, was associated with percent progressive motility, with strongest associations seen for those who were obese in their 20s. Additional life course studies of adiposity and semen quality in larger cohorts are warranted to confirm these results and expand them to include the important developmental periods of puberty and adolescence. In addition, longitudinal studies that collect semen samples at multiple time points beginning in adolescence would provide important information on the natural progression of sperm concentration, motility, and morphology, and provide the opportunity to explore whether interventions such as exercise and weight loss might improve semen quality. Our results provide a compelling reason for clinicians to emphasize the importance of maintaining a healthy body weight both to their adult male patients and to the parents of young boys, as doing so may help prevent a diagnosis of subfertility that can lead to the use of emotionally and financially draining infertility treatment.

Table 2.1. Characteristics of the study sample by current BMI category.

Characteristic	BMI <25 kg/m^2 (n=44)	25≤BMI<30 kg/m^2 (n=78)	BMI ≥30 kg/m^2 (n=67)	p-value	Total (n=193*)
	mean (SD)	mean (SD)	mean (SD)		mean (SD)
Sperm concentration (10^6/mL)	68.2 (53.5)	76.2 (67.6)	73.7 (53.2)	0.78	73.6 (58.8)
% Progressive motility	39.7 (15.8)	42.1 (16.3)	37.7 (15.7)	0.26	40.0 (15.9)
% Normal morphology	6.9 (3.7)	7.7 (4.1)	8.0 (4.7)	0.40	7.6 (4.2)
Age (years)	43.8 (1.6)	43.9 (1.8)	43.8 (1.6)	0.94	43.8 (1.7)
Alcohol (g/week)	88.1 (121.4)	102.5 (160.1)	56.3 (96.1)	0.12	82.3 (131.8)
Caffeine (mg/week)	1539.4 (1699.2)	2223.6 (4781.9)	1446.6 (2032.8)	0.35	1794.7 (3427.9)
Perceived Stress Scale	11.5 (5.6)	11.9 (5.9)	13.6 (6.2)	0.11	12.4 (5.9)
CES-D	27.1 (8.4)	26.5 (7.4)	29.5 (8.2)	0.07	27.6 (8.0)
Exercise (mins/week)	521.7 (871.9)	525.9 (731.9)	571.4 (784.7)	0.93	538.0 (779.7)
Abstinence time (mins)	5806.9 (2614.6)	6009.4 (5853.4)	6804.9 (6009.5)	0.55	6242.7 (5281.1)
	n (percent)	n (percent)	n (percent)		n (percent)
Race				0.76	
White	27 (61.4)	43(55.1)	36(53.7)		110 (567.0)
Black	10 (22.7)	17(21.8)	19(28.4)		46 (23.8)
Other	7 (15.9)	18(23.1)	12(17.9)		37 (19.2)
Education				0.21	
≤High school	9 (20.5)	14 (18.0)	12 (19.4)		35 (18.9)
Some college	11 (25.0)	31 (39.7)	28 (45.2)		71 (38.4)
Bachelors degree	15 (34.1)	23 (29.5)	19 (30.7)		57 (30.8)
Graduate level	9 (20.5)	10 (12.8)	3 (4.8)		22 (11.9)
Income (x US$1,000)				0.56	
≤50	12 (27.3)	21 (28.0)	21 (35.0)		55 (30.6)
50-99	15 (34.1)	26 (34.7)	23 (38.3)		64 (35.6)
100-149	6 (13.6)	16 (21.3)	9 (15.0)		31 (17.2)
≥150	11 (25.0)	12 (16.0)	7 (11.7)		30 (16.7)
Smoking				1.00	
Current	8 (18.2)	14 (18.0)	11 (17.7)		33 (17.8)
Never/ever	36 (81.8)	64 (82.1)	51 (82.3)		152 (82.2)

Hormone use				0.42	
Yes	0 (0)	1 (1.3)	2 (3.3)		3 (1.7)
No	42 (100)	75 (98.7)	59 (96.7)		177 (98.3)
Employment				0.69	
Employed	37 (84.1)	69 (88.5)	52 (83.8)		159 (86.0)
Unemployed	7 (15.9)	9 (11.5)	10 (16.1)		26 (14.1)
STI				1.00	
Any	12 (27.3)	21 (27.3)	17 (27.4)		50 (27.2)
None	32 (72.7)	56 (72.7)	45 (72.6)		134 (72.8)
Adverse Life Events				0.87	
0	21 (47.7)	42 (53.9)	31 (47.0)		95 (50.0)
1	11 (25.0)	20 (25.6)	17 (25.8)		49 (25.8)
2 or more	12 (27.3)	16 (20.5)	18 (27.3)		46 (24.2)
Job strain				0.56	
High	9 (24.3)	13 (18.6)	14 (26.4)		36 (22.2)
Low	28 (75.7)	57 (81.4)	39 (73.6)		126 (77.8)

BMI: body mass index; CES-D: Center for Epidemiologic Studies Depression scale
*includes 4 participants who were missing BMI data

Table 2.2. Associations of bw/ga and adiposity measures with semen outcomes, modeled continuously and dichotomously.

A. Sperm concentration

	Sperm concentration							
	Continuous outcome: square-root sperm concentration (x 10^3/mL)				Dichotomous outcome: <15 million sperm/mL			
Adiposity measure	**Model 1 beta***	**95% CI**	**Model 2 beta***	**95% CI**	**Model 1 OR***	**95% CI**	**Model 2 OR***	**95% CI**
bw/ga	n=193		n=173		n=193		n=173	
continuous	***0.02***	***0.00, 0.04***	***0.02***	***0.00, 0.04***	1.00	0.98, 1.02	1.00	0.98, 1.02
categorical								
≥85 to <95 vs. <85%ile	0.65	-1.03, 2.33	0.8	-1.07, 2.68	†	†	†	†
≥95 vs. <85%ile	***2.90***	***0.11, 5.69***	2.40	-0.77, 5.58	†	†	†	†
wt/age 4 months	n=153		n=140		n=153		n=140	
continuous	0.01	-0.02, 0.03	0.01	-0.02, 0.03	1.01	0.98, 1.04	1.01	0.98, 1.05
categorical								
≥85 to <95 vs. <85%ile	0.78	-1.02, 2.58	0.57	-1.26, 2.41	†	†	†	†
≥95 vs. <85%ile	-0.07	-2.43, 2.30	-0.27	-2.68, 2.13	†	†	†	†
wt/age 12 months	n=152		n=138		n=152		n=138	
continuous	0.01	-0.01, 0.03	0.00	-0.02, 0.03	1.01	0.99, 1.03	1.01	0.99, 1.03
categorical								
≥85 to <95 vs. <85%ile	0.53	-1.55, 2.61	0.14	-1.97, 2.25	0.58	0.06, 5.33	0.91	0.09, 9.48
≥95 vs. <85%ile	-1.82	-4.23, 0.60	-1.99	-4.45, 0.46	**4.77**	**0.70, 32.59**	5.53	0.73, 42.21
ssBMI 4 years	n=160		n=143		n=160		n=143	
continuous	0.00	-0.02, 0.02	0.00	-0.02, 0.02	1.00	0.98, 1.02	0.99	0.97, 1.01
categorical								
≥85 to <95 vs. <85%ile	***-1.64***	***-3.02, -0.26***	***-1.70***	***-3.19, -0.21***	2.12	0.59, 7.69	1.89	0.44, 8.05
≥95 vs. <85%ile	-0.02	-1.62, 1.57	-0.18	-1.82, 1.45	0.59	0.07, 5.32	0.48	0.05, 4.45
BMI in 20s	n=185		n=173		n=185		n=173	
continuous	-0.00	-0.13, 0.13	-0.01	-0.14, 0.13	0.95	0.81, 1.11	0.95	0.80, 1.12

categorical								
≥25 to <30 vs. <25kg/m^2	-0.11	-1.08, 0.85	0.10	-0.89, 1.10	†	†	†	†
≥30 vs. <25 kg/m^2	-1.07	-2.97, 0.84	-1.27	-3.18, 0.64	†	†	†	†
BMI in 30s	n=185		n=173		n=185		n=173	
continuous	-0.07	-0.17, 0.04	-0.04	-0.14, 0.07	0.96	0.85, 1.09	0.92	0.79, 1.06
categorical								
≥25 to <30 vs. <25kg/m^2	0.19	-0.83, 1.21	-0.20	-0.88, 1.27	1.36	0.44, 4.22	1.34	0.38, 4.64
≥30 vs. <25 kg/m^2	-0.58	-1.89, 0.72	-0.38	-1.73, 0.96	0.48	0.08, 2.76	0.27	0.03, 2.41
current BMI	n=189		n=173		n=189		n=173	
continuous	-0.01	-0.10, 0.08	-0.01	-0.10, 0.08	0.92	0.82, 1.02	0.92	0.82, 1.03
categorical								
≥25 to <30 vs. <25kg/m^2	0.09	-1.10, 1.28	-0.12	-1.37, 1.13	0.39	0.11, 1.43	0.42	0.10, 1.70
≥30 vs. <25 kg/m^2	0.08	-1.13, 1.30	0.06	-1.24, 1.36	0.44	0.12, 1.57	0.47	1.12, 1.86

Bw/ga: sex-specific birth weight for gestational age percentile; wt/age: sex-specific weight-for-age percentile; ssBMI: sex-specific BMI percentile; BMI: body mass index

*Model 1: adjusted for bw/ga, age, race, abstinence; Model 2 adjusted for bw/ga, age, race, abstinence, hormones, employment/job strain

† model failed to converge

bold italic: p-value <0.05; **bold:** p-value <0.10

B. Motility

	Progressive motility							
	Continuous outcome: % motile sperm				Dichotomous outcome: <32% progressive motility			
Adiposity measure	**Model 1 beta***	**95% CI**	**Model 2 beta***	**95% CI**	**Model 1 OR***	**95% CI**	**Model 2 OR***	**95% CI**
bw/ga	n=193		n=176		n=193		n=176	
continuous	0.02	-0.06, 0.10	0.03	-0.05, 0.12	1.00	0.98, 1.01	0.99	0.98, 1.01
categorical								
≥85 to <95 vs. <85%ile	3.32	-4.85, 11.48	4.63	-3.92, 12.97	0.50	0.13, 1.96	0.42	0.08, 2.17
≥95 vs. <85%ile	-3.43	-17.00, 10.13	-6.10	-19.29, 7.09	1.56	0.22, 10.88	2.89	0.38, 21.77
wt/age 4 months	n=153		n=140		n=153		n=140	
continuous	0.04	-0.07, 0.15	0.01	-0.11, 0.13	1.00	0.98, 1.01	1.00	0.98, 1.02
categorical								
≥85 to <95 vs. <85%ile	0.62	-7.77, 9.01	-2.91	-11.71, 5.88	0.83	0.24, 2.87	1.14	0.27, 4.77
≥95 vs. <85%ile	-3.92	-14.94, 7.10	-5.23	-16.18, 5.73	1.37	0.29, 6.49	1.41	0.24, 8.46
wt/age 12 months	n=152		n=138		n=152		n=138	
continuous	0.02	-0.07, 0.12	-0.00	-0.10, 0.09	1.00	0.99, 1.02	1.00	0.98, 1.02
categorical								
≥85 to <95 vs. <85%ile	6.62	-2.83, 16.07	2.99	-6.78, 12.75	0.41	0.09, 1.79	0.64	0.11, 3.59
≥95 vs. <85%ile	-1.86	-12.83, 9.11	-5.10	-16.16., 5.96	1.46	0.31, 6.83	2.08	0.36, 12.13
ssBMI 4 years	n=160		n=145		n=160		n=145	
continuous	-0.00	-0.09, 0.09	-0.03	-0.12, 0.07	1.00	0.99, 1.01	1.00	0.98, 1.01
categorical								
≥85 to <95 vs. <85%ile	**-6.14**	**-12.79, 0.51**	***-8.97***	***-16.19, -1.75***	1.45	0.55, 3.78	2.16	0.66, 7.08
≥95 vs. <85%ile	3.57	-4.15, 11.29	3.46	-4.39, 11.31	1.07	0.36, 3.25	1.09	0.30, 3.96
BMI in 20s	n=185		n=176		n=185		n=176	
continuous	-0.35	-0.99, 0.29	**-0.58**	**-1.24, 0.08**	1.07	0.98, 1.18	**1.12**	**0.99, 1.25**
categorical								
≥25 to <30 vs. <25kg/m^2	-3.87	-8.54, 0.80	-3.75	-8.60, 1.10	***2.35***	***1.17, 4.71***	***2.58***	***1.10, 6.01***

≥30 vs. <25 kg/m^2	-4.89	-10.28, 2.39	**-8.08**	**-17.13, 0.97**	2.19	0.58, 8.30	4.24	0.95, 19.02
BMI in 30s	n=185		n=176		n=185		n=176	
continuous	-0.36	-0.87, 0.15	-0.38	-0.89, 0.13	***1.08***	***1.01, 1.17***	**1.09**	**0.99, 1.19**
categorical								
≥25 to <30 vs. <25kg/m^2	-0.46	-4.50, 5.43	-0.27	-5.39, 4.86	1.47	0.69, 3.14	1.43	0.57, 3.55
≥30 vs. <25 kg/m^2	-3.94	-10.28, 2.39	**-5.98**	**-12.46, 0.49**	2.41	0.96, 6.06	***3.18***	***1.05, 9.66***
current BMI	n=189		n=175		n=189		n=175	
continuous	-0.25	-0.69, 0.18	-0.30	-0.74, 0.13	1.04	0.98, 1.11	1.02	0.95, 1.10
categorical								
≥25 to <30 vs. <25kg/m^2	2.20	-3.60, 7.99	1.38	-4.30, 7.05	1.07	0.45, 2.56	0.97	0.37, 2.45
≥30 vs. <25 kg/m^2	-1.46	-7.34, 4.43	-2.95	-8.94, 3.03	1.64	0.69, 3.92	1.40	0.52, 3.79

Bw/ga: sex-specific birth weight for gestational age percentile; wt/age: sex-specific weight-for-age percentile; ssBMI: sex-specific BMI percentile; BMI: body mass index

*Model 1 adjusted for bw/ga, age, race, abstinence; Model 2 adjusted for bw/ga, age, race, abstinence, education, income, smoking, exercise, employment, Perceived Stress Scale, Life Events Inventory, Center for Epidemiologic Studies Depression scale

bold italic: p-value <0.05; **bold:** p-value <0.10

C. Morphology

	Normal morphology							
	Continuous outcome: % normal morphology				Dichotomous outcome: <4% normal morphology			
Adiposity measure	**Model 1 beta***	**95% CI**	**Model 2 beta***	**95% CI**	**Model 1 OR***	**95% CI**	**Model 2 OR***	**95% CI**
bw/ga	n=192		n=176		n=192		n=176	
continuous	-0.00	-0.02, 0.02	0.00	-0.02, 0.03	1.00	0.99, 1.02	1.00	0.99, 1.02
categorical								
≥85 to <95 vs. <85%ile	0.13	-2.06, 2.33	0.54	-1.87, 2.95	†	†	†	†
≥95 vs. <85%ile	**3.14**	**-0.50, 6.79**	2.97	-0.72, 6.65	†	†	†	†
wt/age 4 months	n=152		n=141		n=152		n=141	
continuous	-0.00	-0.03, 0.03	-0.01	-0.05, 0.02	1.01	0.99, 1.03	1.01	0.99, 1.03
categorical								
≥85 to <95 vs. <85%ile	-0.79	-3.12, 1.54	-1.22	-3.78, 1.34	2.11	0.64, 6.98	3.16	0.86, 11.67
≥95 vs. <85%ile	-0.88	-3.94, 2.17	-1.45	-4.81, 1.90	1.99	0.43, 9.31	2.40	0.46, 12.53
wt/age 12 months	n=151		n=138		n=151		n=138	
continuous	0.01	-0.02, 0.03	0.01	-0.02, 0.04	1.00	0.99, 1.02	1.00	0.99, 1.02
categorical								
≥85 to <95 vs. <85%ile	-1.04	-3.59, 1.51	-0.87	-3.57, 1.82	2.09	0.57, 7.67	2.21	0.53, 9.19
≥95 vs. <85%ile	-0.37	-3.32, 2.59	-1.30	-4.59, 1.99	1.74	0.37, 8.09	2.28	0.43, 12.16
ssBMI 4 years	n=159		n=145		n=159		n=145	
continuous	0.01	-0.02, 0.03	0.00	-0.02, 0.03	1.00	0.99, 1.02	1.01	0.99, 1.02
categorical								
≥85 to <95 vs. <85%ile	-0.51	-2.34, 1.32	-0.97	-2.96, 1.02	1.21	0.42, 3.48	1.48	0.46, 4.76
≥95 vs. <85%ile	1.22	-0.88, 3.32	0.49	-1.81, 2.79	0.91	0.26, 3.17	1.15	0.30, 4.41
BMI in 20s	n=184		n=176		n=184		n=176	
continuous	0.10	-0.07, 0.28	0.06	-0.12, 0.24	1.03	0.93, 1.15	1.07	0.96, 1.20
categorical								
≥25 to <30 vs. <25kg/m^2	0.18	-1.11, 1.47	0.01	-1.31, 1.33	1.69	0.80, 3.59	2.13	0.94, 4.80

≥30 vs. <25 kg/m^2	-0.29	-2.82, 2.24	-0.99	-3.63, 1.66	1.97	0.45, 8.71	3.51	0.72, 17.11
BMI in 30s	n=184		n=176		n=184		n=176	
continuous	0.02	-0.12, 0.16	0.00	-0.14, 0.15	1.06	0.98, 1.15	1.08	0.99, 1.18
categorical								
≥25 to <30 vs. <25kg/m^2	0.84	-0.52, 2.19	0.39	-1.03, 1.81	1.11	0.49, 2.53	1.54	0.63, 3.75
≥30 vs. <25 kg/m^2	0.67	-1.07, 2.40	0.56	-1.23, 2.36	1.64	0.60, 4.50	2.16	0.72, 6.47
current BMI	n=188		n=175		n=188		n=175	
continuous	0.06	-0.06, 0.18	0.06	-0.06, 0.19	1.04	0.97, 1.12	1.06	0.98, 1.14
categorical								
≥25 to <30 vs. <25kg/m^2	0.75	-0.82, 2.32	0.62	-0.98, 2.23	0.78	0.29, 2.11	0.83	0.29, 2.36
≥30 vs. <25 kg/m^2	1.20	-0.39, 2.79	1.08	-0.58, 2.74	1.53	0.59, 3.95	**1.91**	**0.70, 5.27**

Bw/ga: sex-specific birth weight for gestational age percentile; wt/age: sex-specific weight-for-age percentile; ssBMI: sex-specific BMI percentile; BMI: body mass index

*Model 1 adjusted for bw/ga, age, race, abstinence; Model 2 adjusted for bw/ga, age, race, abstinence, smoking, alcohol, employment, Perceived Stress Scale, Life Events Inventory

† model failed to converge

bold italic: p-value <0.05; **bold:** p-value <0.10

Table 2.3. Critical period models regressing semen parameters on adiposity measures.
A. Sperm concentration

Square-root sperm concentration (x 10^3/mL)				
	Model 1 beta*	**95% CI**	**Model 2 beta***	**95% CI**
Model 1	n=153		n=140	
wt/ht 4 months	0.00	-0.02, 0.02	-0.01	-0.03, 0.01
bw/ga	0.01	-0.01, 0.03	0.01	-0.01, 0.03
Model 2	n=142		n=129	
wt/ht 12 months	0.02	-0.01, 0.04	0.02	-0.00, 0.05
wt/ht 4 months	-0.01	-0.04, 0.02	**-0.03**	**-0.05, 0.00**
bw/ga	0.01	-0.01, 0.03	0.01	-0.01, 0.03
Model 3	n=132		n=120	
ssBMI 4 years	-0.01	-0.04, 0.02	-0.01	-0.04, 0.02
wt/ht 12 months	0.02	-0.01, 0.05	**0.03**	**-0.00, 0.05**
wt/ht 4 months	-0.00	-0.03, 0.02	-0.02	-0.05, 0.01
bw/ga	0.00	-0.02, 0.03	0.01	-0.01, 0.03
Model 4	n=128		n=120	
BMI 20s	-0.01	-0.19, 0.18	-0.01	-0.20, 0.17
ssBMI 4 years	-0.01	-0.04, 0.02	-0.01	-0.04, 0.02
wt/ht 12 months	0.02	-0.01, 0.05	**0.03**	**-0.00, 0.05**
wt/ht 4 months	-0.00	-0.03, 0.02	-0.02	-0.05, 0.01
bw/ga	0.01	-0.02, 0.03	0.01	-0.01, 0.03
Model 5	n=128		n=120	
BMI 30s	-0.12	-0.36, 0.12	-0.07	-0.32, 0.17
BMI 20s	0.12	-0.20, 0.44	0.06	-0.26, 0.39
ssBMI 4 years	-0.01	-0.04, 0.02	-0.01	-0.04, 0.02
wt/ht 12 months	0.02	-0.01, 0.05	**0.03**	**-0.00, 0.05**
wt/ht 4 months	-0.01	-0.04, 0.02	-0.02	-0.05, 0.01
bw/ga	0.01	-0.02, 0.03	0.01	-0.01, 0.03
Model 6	n=127		n=119	
current BMI	0.09	-0.09, 0.27	0.01	-0.18, 0.20
BMI 30s	-0.18	-0.45, 0.09	-0.08	-0.35, 0.20
BMI 20s	0.09	-0.24, 0.41	0.06	-0.27, 0.39
ssBMI 4 years	-0.01	-0.04, 0.02	-0.01	-0.04, 0.02
wt/ht 12 months	0.02	-0.01, 0.05	0.02	-0.01, 0.06
wt/ht 4 months	-0.01	-0.04, 0.02	-0.02	-0.05, 0.01
bw/ga	0.01	-0.01, 0.03	0.01	-0.01, 0.03

Bw/ga: sex-specific birth weight for gestational age percentile; wt/ht: sex-specific height-for-age percentile; ssBMI: sex-specific BMI percentile; BMI: body mass index
*Model 1 covariate-unadjusted; Model 2 adjusted for age, race, abstinence, hormones, employment/job strain, age (days) at 4 months, age (days) at 12 months
bold italic: p-value <0.05; **bold:** p-value <0.10

B. Motility

% Progressive motility				
	Model 1 beta*	**95% CI**	**Model 2 beta***	**95% CI**
Model 1	n=153		n=140	
wt/ht 4 months	-0.02	-0.12, 0.07	-0.05	-0.15, 0.05
bw/ga	0.02	-0.06, 0.11	0.03	-0.07, 0.13
Model 2	n=142		n=129	
wt/ht 12 months	-0.06	-0.17, 0.06	-0.04	-0.15, 0.08
wt/ht 4 months	0.02	-0.10, 0.14	-0.03	-0.15, 0.09
bw/ga	0.02	-0.07, 0.12	0.02	-0.08, 0.12
Model 3	n=132		n=120	
ssBMI 4 years	0.01	-0.12, 0.14	-0.02	-0.15, 0.11
wt/ht 12 months	-0.04	-0.18, 0.09	-0.01	-0.15, 0.13
wt/ht 4 months	0.05	-0.08, 0.17	0.00	-0.13, 0.13
bw/ga	0.01	-0.09, 0.11	0.01	-0.10, 0.12
Model 4	n=128		n=122	
BMI 20s	-0.27	-1.14, 0.59	-0.16	-1.05, 0.74
ssBMI 4 years	0.02	-0.12, 0.16	-0.01	-0.16, 0.13
wt/ht 12 months	-0.04	-0.18, 0.10	-0.01	-0.15, 0.13
wt/ht 4 months	0.05	-0.09, 0.18	-0.00	-0.13, 0.13
bw/ga	0.02	-0.08, 0.12	0.01	-0.10, 0.12
Model 5	n=128		n=122	
BMI 30s	-0.23	-1.37, 0.91	-0.07	-1.26, 1.11
BMI 20s	-0.03	-1.52, 1.46	-0.08	-1.64, 1.49
ssBMI 4 years	0.02	-0.12, 0.16	-0.01	-0.16, 0.13
wt/ht 12 months	-0.05	-0.19, 0.09	-0.01	-0.15, 0.13
wt/ht 4 months	0.04	-0.09, 0.18	-0.00	-0.13, 0.1
bw/ga	0.02	-0.08, 0.13	0.01	-0.10, 0.12
Model 6	n=127		n=119	
current BMI	-0.01	-0.84, 0.83	0.11	-0.73, 0.95
BMI 30s	-0.22	-1.49, 1.05	-0.15	-1.47, 1.17
BMI 20s	-0.03	-1.56, 1.50	-0.11	-1.72, 1.50
ssBMI 4 years	0.02	-0.12, 0.16	-0.01	-0.16, 0.13
wt/ht 12 months	-0.05	-0.19, 0.10	-0.02	-0.16, 0.13
wt/ht 4 months	0.04	-0.10, 0.17	-0.00	-0.14, 0.13
bw/ga	0.03	-0.08, 0.13	0.01	-0.10, 0.12

Bw/ga: sex-specific birth weight for gestational age percentile; wt/ht: sex-specific height-for-age percentile; ssBMI: sex-specific BMI percentile; BMI: body mass index

*Model 1 covariate-unadjusted; Model 2 adjusted for age, race, abstinence, education, income, smoking, exercise, employment, Perceived Stress Scale, Life Events Inventory, Center for Epidemiologic Studies Depression scale, age (days) at 4 months, age (days) at 12 months

C. Morphology

% Normal morphology				
	Model 1 beta*	**95% CI**	**Model 2 beta***	**95% CI**
Model 1	n=152		n=141	
wt/ht 4 months	0.01	-0.02, 0.03	-0.00	-0.03, 0.02
bw/ga	-0.01	-0.04, 0.01	-0.01	-0.04, 0.02
Model 2	n=141		n=130	
wt/ht 12 months	0.01	-0.03, 0.04	0.02	-0.01, 0.05
wt/ht 4 months	0.00	-0.03, 0.04	-0.02	-0.05, 0.02
bw/ga	-0.01	-0.04, 0.01	-0.01	-0.04, 0.02
Model 3	n=131		n=121	
ssBMI 4 years	-0.00	-0.04, 0.03	-0.01	-0.04, 0.03
wt/ht 12 months	0.00	-0.03, 0.04	0.02	-0.02, 0.06
wt/ht 4 months	0.01	-0.03, 0.04	-0.01	-0.05, 0.02
bw/ga	-0.01	-0.04, 0.01	-0.01	-0.04, 0.02
Model 4	n=127		n=121	
BMI 20s	**0.22**	**-0.00, 0.44**	0.18	0.04, 0.41
ssBMI 4 years	-0.01	-0.05, 0.02	-0.02	-0.05, 0.02
wt/ht 12 months	0.01	-0.03, 0.04	0.02	-0.02, 0.06
wt/ht 4 months	0.01	-0.03, 0.04	-0.01	-0.05, 0.03
bw/ga	-0.01	-0.04, 0.01	-0.01	-0.04, 0.02
Model 5	n=127		n=121	
BMI 30s	-0.06	-0.36, 0.24	-0.03	-0.35, 0.28
BMI 20s	0.28	-0.10, 0.67	0.22	-0.19 0.62
ssBMI 4 years	-0.01	-0.05, 0.03	-0.01	-0.05, 0.02
wt/ht 12 months	0.01	-0.03, 0.04	0.02	-0.02, 0.06
wt/ht 4 months	0.01	-0.03, 0.04	-0.01	-0.05, 0.03
bw/ga	-0.01	-0.04, 0.01	-0.01	-0.04, 0.02
Model 6	n=126		n=120	
current BMI	0.16	-0.06, 0.37	**0.21**	**-0.01, 0.43**
BMI 30s	-0.16	-0.49, 0.16	-0.17	-0.51, 0.17
BMI 20s	0.23	-0.17, 0.62	0.15	-0.26, 0.56
ssBMI 4 years	-0.01	-0.05, 0.03	-0.01	-0.05, 0.02
wt/ht 12 months	0.00	-0.04, 0.04	0.02	-0.02, 0.05
wt/ht 4 months	0.01	-0.02, 0.05	-0.01	-0.04, 0.03
bw/ga	-0.01	-0.04, 0.01	-0.01	-0.04, 0.02

Bw/ga: sex-specific birth weight for gestational age percentile; wt/ht: sex-specific height-for-age percentile; ssBMI: sex-specific BMI percentile; BMI: body mass index
*Model 1 covariate-unadjusted; Model 2 adjusted for age, race, abstinence, smoking, alcohol, employment, Perceived Stress Scale, Life Events Inventory, age (days) 4 months, age (days) 12 months
bold: p-value <0.10

Table 2.4. Linear and logistic regression of semen parameters on cumulative adiposity measures.

A. Sperm concentration

	Sperm concentration											
	Continuous outcome: square-root sperm concentration (x 10^3/mL)						Dichotomous outcome: <15 million sperm/mL					
	n	Model 1 beta*	95% CI	n	Model 2 beta*	95% CI	n	Model 1 OR*	95% CI	n	Model 2 OR*	95% CI
Total overweight/obese points	127	-0.07	-0.43, 0.28	119	-0.15	-0.50, 0.21	127	1.12	0.79, 1.58	119	1.11	0.72, 1.73
Total obese points	127	-0.18	-0.71, 0.34	119	-0.17	-0.69, 0.33	127	1.08	0.66, 1.77	119	1.07	0.60, 1.93
Child overweight/obese points (≥85%ile)	132	-0.25	-0.81, 0.31	120	-0.47	-1.04, 0.10	132	1.34	0.79, 2.25	120	1.38	0.73, 2.63
Child obese points (≥95%ile)	132	-0.48	-1.28, 0.33	120	-0.44	-1.24, 0.36	132	1.39	0.70, 2.74	120	1.32	0.59, 2.99
Adult overweight/obese points (BMI ≥25 kg/m^2)	184	-0.03	-0.42, 0.37	172	-0.02	-0.42, 0.38	184	0.93	0.63, 1.39	172	0.89	0.57, 1.39
Adult obese points (BMI ≥30 kg/m^2)	184	-0.10	-0.63, 0.42	172	-0.15	-0.67, 0.38	184	0.77	0.42, 1.43	172	0.64	0.30, 1.36
Adult overweight/obese points (BMI ≥25 kg/m^2)**	127	0.09	-0.42, 0.61	119	0.07	-0.45. 0.60	127	0.99	0.60, 1.62	119	0.94	0.51, 1.72
Adult obese points (BMI ≥30 kg/m^2)**	127	0.03	-0.65, 0.71	119	-0.01	-0.68, 0.67	127	0.88	0.43, 1.80	119	0.87	0.34, 2.18

*Model 1 adjusted for sex-specific birth weight for gestational age percentile (bw/ga); Model 2 adjusted for bw/ga, age, race, abstinence, hormones, employment/job strain

**restricted to participants with childhood adiposity measures

bold italic: p-value <0.05; **bold:** p-value <0.10

B. Motility

	Progressive motility											
	Continuous outcome: % progressive motility						Dichotomous outcome: <32% progressive motility					
	n	Model 1 beta*	95% CI	n	Model 2 beta*	95% CI	n	Model 1 OR*	95% CI	n	Model 2 OR*	95% CI
Total overweight/obese points	127	-0.39	-2.04, 1.27	119	-0.21	-1.81, 1.38	127	1.15	0.93, 1.43	119	1.15	0.88, 1.50
Total obese points	127	-0.67	-3.11, 1.77	119	-0.72	-3.05, 1.61	127	1.08	0.79, 1.47	119	1.03	0.69, 1.53
Child overweight/obese points (≥85%ile)	132	-0.42	-3.04, 2.20	120	-1.04	-3.68, 1.60	132	1.13	0.81, 1.59	120	1.22	0.78, 1.92
Child obese points (≥95%ile)	132	0.21	-3.53, 3.94	120	0.27	-3.46, 4.01	132	0.94	0.58, 1.54	120	0.89	0.47, 1.70
Adult overweight/obese points (BMI ≥25 kg/m^2)	184	-1.01	-2.98, 0.96	175	-1.19	-3.10, 0.73	184	**1.30**	**0.99, 1.71**	175	**1.33**	**0.96, 1.86**
Adult obese points (BMI ≥30 kg/m^2)	184	-1.95	-4.55, 0.66	175	***-2.60***	***-5.09, -0.12***	184	1.30	0.93, 1.82	175	1.41	0.93, 2.13
Adult overweight/obese points (BMI ≥25 kg/m^2)**	127	-0.46	-2.86, 1.94	119	0.36	-2.10, 2.82	127	1.25	0.91, 1.72	119	1.17	0.78, 1.75
Adult obese points (BMI ≥30 kg/m^2)**	127	-1.13	-4.29, 2.03	119	-1.48	-4.57, 1.61	127	1.17	0.78, 1.74	119	1.15	0.68, 1.96

*Model 1 adjusted for sex-specific birth weight for gestational age percentile (bw/ga); Model 2 adjusted for bw/ga, age, race, abstinence, education, income, smoking, exercise, employment, Perceived Stress Scale, Life Events Inventory, Center for Epidemiologic Studies Depression scale

**restricted to participants with childhood adiposity measures

bold italic: p-value <0.05; **bold:** p-value <0.10

C. Morphology

	Morphology											
	Continuous outcome: % normal morphology						Dichotomous outcome: <4% normal morphology					
	n	Model 1 beta*	95% CI	n	Model 2 beta*	95% CI	n	Model 1 OR*	95% CI	n	Model 2 OR*	95% CI
Total overweight/obese points	126	0.34	-0.10, 0.77	120	0.26	-0.19, 0.72	126	1.1	0.86, 1.42	120	1.16	0.87, 1.54
Total obese points	126	0.3	-0.34, 0.94	120	0.26	-0.40, 0.91	126	1.2	0.84, 1.70	120	1.25	0.84, 1.85
Child overweight/obese points (≥85%ile)	131	0.29	-0.41, 0.99	121	0.08	-0.67, 0.84	131	1.03	0.69, 1.53	121	1.10	0.70, 1.70
Child obese points (≥95%ile)	131	-0.28	-1.27, 0.70	121	-0.45	-1.54, 0.64	131	1.24	0.73, 2.09	121	1.23	0.67, 2.25
Adult overweight/obese points (BMI ≥25 kg/m^2)	183	0.22	-0.30, 0.74	175	0.14	-0.39, 0.66	183	1.19	0.87, 1.61	175	**1.34**	**0.95, 1.89**
Adult obese points (BMI ≥30 kg/m^2)	183	0.22	-0.48, 0.91	175	0.17	-0.53, 0.87	183	1.28	0.88, 1.86	175	**1.48**	**0.97, 2.26**
Adult overweight/obese points (BMI ≥25 kg/m^2)**	126	0.51	-0.12, 1.13	120	0.49	-0.15, 1.13	126	1.14	0.79, 1.64	120	1.23	0.81, 1.87
Adult obese points (BMI ≥30 kg/m^2)**	126	0.73	-0.09, 1.5	120	0.7	-0.13, 1.53	126	1.14	0.72, 1.81	120	1.25	0.73, 2.12

*Model 1 adjusted for sex-specific birth weight for gestational age percentile (bw/ga); Model 2 adjusted for bw/ga, age, race, abstinence, smoking, alcohol, employment, Perceived Stress Scale, Life Events Inventory
**restricted to participants with childhood adiposity measures
bold: p-value <0.10

Table 2.5. Trajectories of adiposity change in childhood and adulthood as predictors of semen quality in middle age.
A. Sperm concentration

	Sperm concentration							
	Continuous outcome: square-root sperm concentration (x 10^3/mL)				Dichotomous outcome: <15 million sperm/mL			
	Model 1 beta*	95% CI	Model 2 beta*	95% CI	Model 1 OR*	95% CI	Model 2 OR*	95% CI
Childhood trajectory	n=140		n=128		n=140		n=128	
Gained vs. maintained	-1.09	-2.59, 0.41	-0.52	-2.09, 1.06	2.49	0.66, 9.45	1.55	0.31. 7.84
Lost vs. maintained	0.06	-1.66, 1.78	-0.60	-2.41, 1.21	1.74	0.33, 9.22	2.82	0.34, 23.19
Adult trajectory	n=179		n=168		n=179		n=168	
Gained from overweight or obese vs. maintained normal	0.07	-1.28, 1.41	0.16	-1.21, 1.54	0.81	0.21, 3.04	0.55	0.12, 2.53
Gained from normal vs. maintained normal	0.25	-1.05, 1.55	-0.03	-1.36, 1.30	0.52	0.13, 2.10	0.41	0.08, 2.05
Maintained overweight or obese vs. maintained normal	-0.31	-1.88, 1.26	-0.40	-1.96, 1.16	0.87	0.19, 4.07	0.75	0.14, 4.13
Lifetime trajectory	n=138		n=125		n=138		n=125	
Normal child/overweight or obese adult vs. always normal	0.31	-1.54, 2.17	0.15	-1.73, 2.03	†	†	†	†
Overweight or obese child/normal adult vs. always normal	0.71	-1.99, 3.41	0.73	-2.05, 3.52	†	†	†	†
Always overweight or obese vs. always normal	0.31	-1.45, 2.07	-0.24	-2.02, 1.54	†	†	†	†
Normal or overweight child/obese adult vs. never obese	0.34	-1.10, 1.77	0.45	-1.09, 1.99	0.72	0.13, 3.95	0.47	0.05, 4.92
Obese child/normal or overweight adult vs. never obese	-1.28	-2.80, 0.23	-1.08	2.64, 0.48	1.74	0.42, 7.17	0.73	0.12, 4.33
Always obese vs. never obese	-0.24	-2.09, 1.61	-0.24	-2.08, 1.60	1.53	0.27, 8.82	1.51	0.22, 10.17

*Model 1: adjusted for sex-specific birth weight for gestational age percentile (bw/ga); Model 2: adjusted for bw/ga, age, race, abstinence, hormones, employment/job strain

B. Motility

	Progressive motility							
	Continuous outcome: % progressive motility				Dichotomous outcome: <32% progressive motility			
	Model 1 beta*	95% CI	Model 2 beta*	95% CI	Model 1 OR*	95% CI	Model 2 OR*	95% CI
Childhood trajectory	n=140		n=128		n=140		n=128	
Gained vs. maintained	-2.65	-9.75, 4.45	-2.67	-10.16, 4.82	1.55	0.63, 3.82	2.64	0.80, 8.75
Lost vs. maintained	1.46	-6.73, 9.66	-2.77	-11.47, 5.94	0.76	0.25, 2.34	1.00	0.21, 4.67
Adult trajectory	n=184		n=175		n=184		n=175	
Gained from overweight or obese vs. maintained normal	-3.61	-10.23, 3.00	-2.97	-9.29, 3.35	1.98	0.81, 4.81	1.66	0.57, 4.88
Gained from normal vs. maintained normal	2.47	-3.89, 8.83	1.52	-4.54, 7.57	0.83	0.33, 2.07	0.62	0.21, 1.87
Maintained overweight or obese vs. maintained normal	-2.38	-10.03, 5.28	-3.39	-11.01, 4.22	2.05	0.74, 5.66	2.96	0.85, 10.29
Lifetime trajectory	n=137		n=125		n=137		n=125	
Normal child/overweight or obese adult vs. always normal	-6.50	-14.46, 1.46	-3.72	-11.97, 4.53	2.05	0.72, 5.83	0.97	0.22, 4.35
Overweight or obese child/normal adult vs. always normal	-2.70	-9.29, 3.88	-2.78	-9.42, 3.86	1.35	0.56, 3.30	1.46	0.47, 4.52
Always overweight or obese vs. always normal	-2.33	-9.98, 5.24	-2.57	-10.46, 5.33	1.53	0.56, 4.21	1.39	0.33, 5.85
Normal or overweight child/obese adult vs. never obese	-2.88	-9.65, 3.86	-0.95	-8.48, 6.58	1.41	0.59, 3.39	0.60	0.14, 2.58
Obese child/normal or overweight adult vs. never obese	-0.28	-7.41, 6.85	0.11	-6.76, 6.98	0.74	0.28, 1.98	0.58	0.17, 1.98
Always obese vs. never obese	-4.73	-13.44, 3.97	-4.47	-13.34, 4.40	1.82	0.60, 5.55	1.79	0.41, 7.87

*Model 1: adjusted for sex-specific birth weight for gestational age percentile (bw/ga); Model 2: adjusted for bw/ga, age, race, abstinence, education, income, smoking, exercise, employment, Perceived Stress Scale, Life Events Inventory, Center for Epidemiologic Studies Depression scale

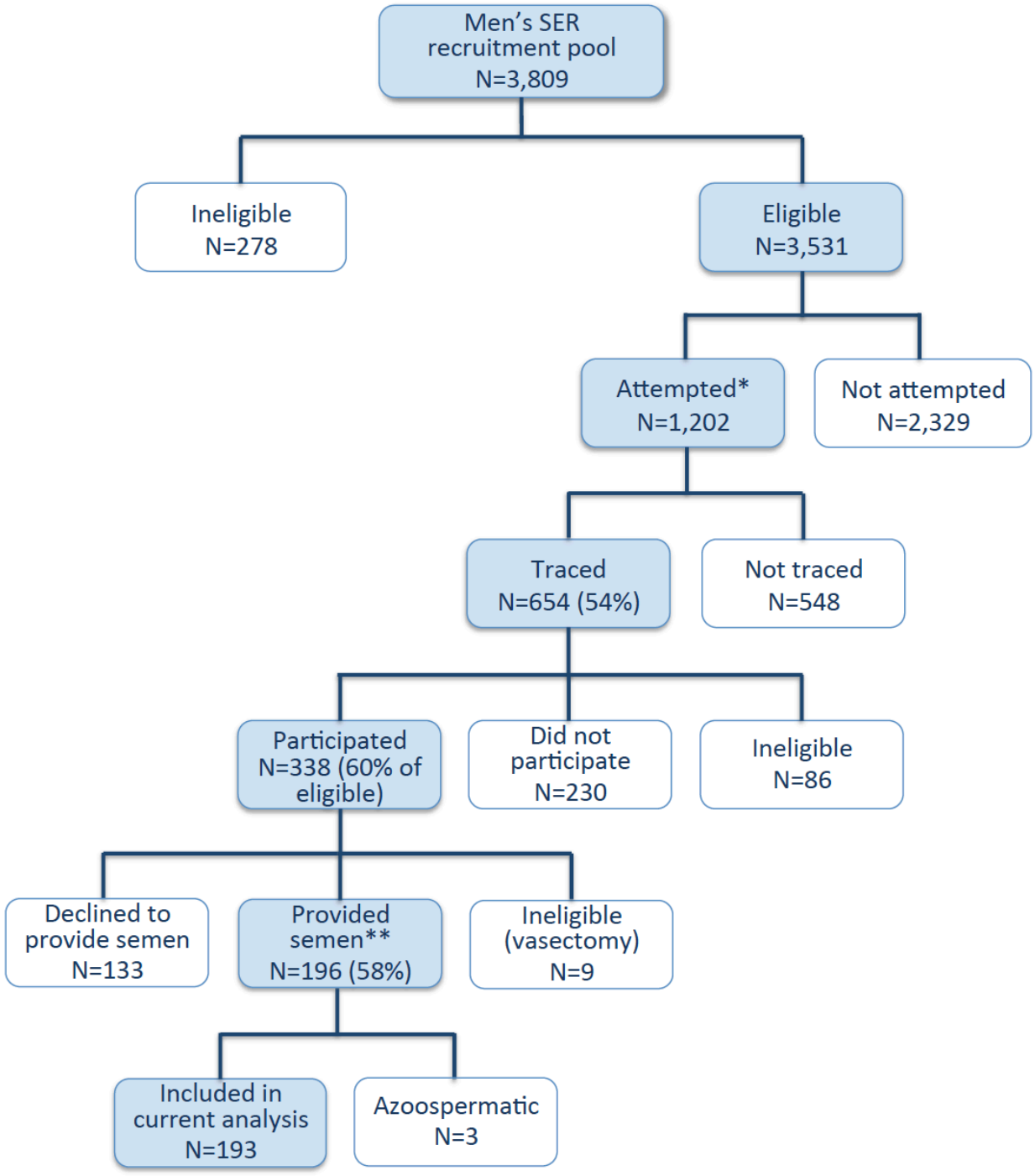
C. Morphology

	Morphology							
	Continuous outcome: % normal morphology				Dichotomous outcome: <4% normal morphology			
	Model 1 beta*	95% CI	Model 2 beta*	95% CI	Model 1 OR*	95% CI	Model 2 OR*	95% CI
Childhood trajectory	n=139		n=129		n=139		n=129	
Gained vs. maintained	0.12	-1.82, 2.06	0.05	-1.99, 2.09	0.91	0.32, 2.59	0.77	0.24, 2.54
Lost vs. maintained	1.46	-0.82, 3.74	1.02	-1.46, 3.51	0.42	0.09, 1.98	0.49	0.09, 2.71
Adult trajectory	n=178		n=171		n=178		n=171	
Gained from overweight or obese vs. maintained normal	0.90	-0.87, 2.68	0.82	-0.96, 2.60	1.97	0.71, 5.49	2.38	0.78, 7.25
Gained from normal vs. maintained normal	1.16	-0.56, 2.88	0.95	-0.76, 2.66	0.93	0.32, 2.71	0.90	0.28, 2.94
Maintained overweight or obese vs. maintained normal	-0.05	-2.12, 2.02	-0.11	-2.16, 1.94	1.51	0.46, 4.97	1.80	0.50, 6.54
Lifetime trajectory	n=137		n=128		n=137		n=128	
Normal child/overweight or obese adult vs. always normal	1.43	-0.84, 3.70	1.9	-0.43, 4.23	0.75	0.21, 2.69	0.59	0.14, 2.46
Overweight or obese child/normal adult vs. always normal	1.84	-1.47, 5.16	1.68	-1.66, 5.01	0.29	0.03, 3.04	0.32	0.03, 3.53
Always overweight or obese vs. always normal	2.03	-0.13, 4.19	1.68	-0.56, 3.92	0.70	0.21, 2.35	0.80	0.22, 2.96
Normal or overweight child/obese adult vs. never obese	***2.70***	***0.95, 4.45***	***3.52***	***1.68. 5.36***	0.99	0.35, 2.81	0.89	0.26, 3.11
Obese child/normal or overweight adult vs. never obese	0.44	-1.43, 2.31	0.66	-1.22. 2.54	1.19	0.41, 3.46	1.01	0.32, 3.21
Always obese vs. never obese	0.81	-1.45, 3.06	0.73	-1.62, 3.08	2.12	0.64, 6.98	2.18	0.58, 8.21

*Model 1: adjusted for sex-specific birth weight for gestational age percentile (bw/ga); Model 2 adjusted for bw/ga, age, race, abstinence, smoking, alcohol, employment, Perceived Stress Scale, Life Events Inventory

bold ital: p-value <0.05

Figure 2.1. Participants in the SER follow-up to the CHDS included in the current analysis.



SER: Study of the Environment and Reproduction; CHDS: Child Health and Development Studies
*Attempted contact
**160 participants provided two samples, 36 provided one

Supplemental Table 2.1. Comparison of CHDS and SER cohorts.*

		Male CHDS offspring		SER non-participants		SER participants without semen samples		SER participants with semen samples	p-value comparing SER participants with and without semen samples	p-value comparing SER participants with semen samples to male CHDS offspring
	n	mean (SD)	n	mean (SD)	n	mean (SD)	n	mean (SD)		
Gestational age (weeks)	8602	40.0 (1.8)	8290	40.0 (1.8)	139	40.4 (1.6)	196	39.8 (2.4)	0.02	0.42
Birth weight (g)	8602	3438.2 (485.3)	8290	3433.7 (480.8)	142	3465.4 (544.3)	196	3388.3 (535.2)	0.20	0.20
	n	%	n	%	n	%	n	%		
Maternal race	8504		8166		142		196			
White	5795	68.1	5571	68.2	102	71.8	122	62.2	0.07	0.08
Black	1861	21.9	1791	21.9	25	17.6	45	23.0	0.23	0.72
Hispanic	273	3.2	264	3.2	1	0.7	8	4.1	0.06	0.50
Asian	342	4.0	314	3.9	11	7.8	17	8.7	0.76	0.001
Other	233	2.7	226	2.8	3	2.1	4	2.0	0.96	0.55

CHDS: Child Health and Development Studies; SER: Study of the Environment and Reproduction
*Excludes those with gestational age <37 weeks or major congenital abnormalities
† n=8,602 for gestational age and birth weight and 8,504 for maternal race
†† n=8,290 for gestational age and birth weight and 8,166 for maternal race
††† n=139 for gestational age and 142 for birth weight and maternal race
†††† n=196

Supplemental Table 2.2: Creation of childhood and adulthood trajectory variables.

In order to determine categories for the child and adult trajectory analyses, we first determined how many participants changed adiposity category between 4 months and 4 years, or between their 20s and when the Study of the Environment and Reproduction was conducted.

Changes in child adiposity levels using weight-for-height percentile		**Changes in adult BMI levels**	
Category	**n**	**Category**	**n**
normal-->normal	90	normal-->overweight	50
overweight-->overweight	8	normal-->obese	6
obese-->obese	0	normal-->morbidly obese	4
normal-->overweight	21	overweight-->obese	32
normal-->obese	0	overweight-->morbidly obese	10
overweight-->obese	0	obese-->morbidly obese	8
obese-->overweight	9	normal-->normal	41
obese-->normal	4	overweight-->overweight	26
overweight-->normal	8	obese-->obese	1
		morbidly obese-->morbidly obese	1
		morbidly obese-->obese	0
		morbidly obese-->overweight	0
		morbidly obese-->normal	0
		obese-->overweight	2
		obese-->normal	0
		overweight-->normal	3
Total	140	Total	184
Normal: <85%ile; overweight: ≥85 and <95%ile; obese: ≥95%ile		Normal: BMI <25 kg/m^2; overweight: BMI ≥25 and <30 kg/m^2; obese: BMI ≥30 and <35 kg/m^2; morbidly obese: ≥35 kg/m^2	

BMI: body mass index

We then grouped the child adiposity trajectories into three categories: maintained (yellow), increased (pink), and reduced (green), with maintained used as the reference category in regression analysis. We also grouped the adult adiposity trajectories into three categories. The reference category was those who maintained a normal BMI (yellow). The contrasting categories were those who had normal BMI in their 20s but >normal at the time of the study (blue); those who were overweight or obese in their 20s and became obese or morbidly obese, respectively (pink); and those who had >normal BMI in their 20s and either maintained or reduced (green). Results did not change when we performed sensitivity analyses of the adult trajectories without the 5 participants who reduced.

Supplemental Table 2.3. Semen outcomes by levels of potential covariates.

Characteristic	Sperm concentration (x 10^6/mL)		% Progressive motility		% Normal morphology	
	n	mean (SD)	n	mean (SD)	n	mean (SD)
Race				*		*
White	110	73.2 (52.7)	110	41.3 (16.2)	109	7.3 (4.1)
Black	46	68.5 (57.5)	46	32.3 (14.5)	46	6.6 (3.9)
Other	37	81.4 (76.2)	37	43.3 (15.1)	37	9.5 (4.2)
Education						
≤High school	35	92.8 (87.4)	35	34.0 (17.0)	35	8.0 (4.7)
Some college	71	74.2 (55.2)	71	41.9 (15.6)	71	7.5 (4.4)
Bachelors degree	57	59.9 (40.3)	57	40.4 (13.9)	57	7.4 (3.9)
Graduate level	22	73.9 (57.1)	22	42.3 (19.2)	21	7.7 (4.2)
Income (x US$1,000)				*		
≤ 50	55	81.1 (77.5)	55	35.5 (17.4)	55	7.2 (4.5)
50-99	64	74.7 (43.0)	64	41.6 (15.6)	63	8.2 (4.2)
100-149	31	62.8 (58.5)	31	45.0 (16.3)	31	7.6 (4.5)
≥ 150	30	57.0 (35.5)	30	41.3 (12.8)	30	7.0 (3.7)
Smoking				*		
Current	33	83.6 (86.9)	33	30.8 (15.1)	33	6.3 (3.9)
Never/ever	152	71.0 (52.1)	152	42.0 (15.5)	151	7.9 (4.3)
Employment status				*		*
Employed	159	75.9 (59.0)	159	41.7 (15.3)	158	7.9 (4.2)
Unemployed	26	57.4 (63.1)	26	29.2 (15.9)	26	5.9 (4.3)
Hormone use						
Yes	177	74.5 (60.2)	177	40.0 (16.0)	176	7.7 (4.2)
No	3	22.2 (9.3)	3	45.7 (2.5)	3	3.8 (1.4)
Life Events Inventory				*		*
0	95	70.8 (53.7)	95	42.5 (15.3)	94	8.3 (4.1)
1	49	78.9 (46.8)	49	42.1 (16.3)	49	7.4 (3.7)
2 or more	46	74.7 (79.2)	46	33.2 (15.1)	46	6.4 (4.7)
Job strain						
Low	126	76.5 (62.9)	126	42.6 (15.3)	125	7.9 (4.3)
High	36	75.6 (51.1)	36	39.7 (15.7)	36	7.3 (3.7)
		corr		**corr**		**corr**
Age (years)	193	-0.20*	193	-0.26*	192	-0.20*
Alcohol (g/week)	182	0.01	182	0.06	181	0.004
Caffeine (mg/week)	185	0.07	185	0.13	184	-0.07
Exercise (minutes/week)	188	-0.01	188	-0.13	187	-0.08
Perceived Stress Scale	190	-0.11	190	-0.18*	189	-0.19*
CES-D	188	-0.06	188	-0.23*	187	-0.11
Abstinence time (minutes)	193	0.19*	193	-0.08	192	0.05

CES-D: Center for Epidemiologic Studies Depression scale; corr: Spearman correlation coefficient
*p-value <0.05

Supplemental Table 2.4. Spearman correlations among semen parameters.

Semen outcome	1	2	3
1 Sperm concentration			
correlation	1		
p-value			
n	193		
2 % Progressive motility			
correlation	0.37	1	
p-value	<0.0001		
n	193	193	
3 % Normal morphology			
correlation	0.52	0.43	1
p-value	<0.0001	<0.0001	
n	192	192	192

Supplemental Table 2.5. Spearman correlations among bw/ga and adiposity measures.

	1	2	3	4	5	6	7
1 bw/ga							
correlation	1						
p-value							
n	193						
2 wt/age 4 months							
correlation	0.47	1					
p-value	<.0001						
n	153	153					
3 wt/age 1 year							
correlation	0.40	0.67	1				
p-value	<.0001	<.0001					
n	152	142	152				
4 ssBMI 4 years							
correlation	0.15	0.35	0.49	1			
p-value	0.05	<.0001	<.0001				
n	160	140	141	160			
5 BMI in 20s							
correlation	0.08	0.05	0.11	0.23	1		
p-value	0.27	0.53	0.18	0.004			
n	185	148	146	153	185		
6 BMI in 30s							
correlation	0.09	0.08	0.10	0.19	0.84	1	
p-value	0.24	0.31	0.22	0.02	<.0001		
n	185	148	146	153	185	185	
7 current BMI							
correlation	0.04	0.12	0.16	0.21	0.69	0.80	1
p-value	0.54	0.15	0.06	0.01	<.0001	<.0001	
n	189	150	148	157	184	184	189

Bw/ga: sex-specific birth weight for gestational age percentile; wt/ht: sex-specific height-for-age percentile; ssBMI: sex-specific BMI percentile; BMI: body mass index

Supplemental Table 2.6. Multinomial logistic regression of number of WHO subfertility criteria by continuous and dichotomous adiposity measures.

	2 or 3 WHO subfertility criteria vs. none				1 WHO criterion vs. none			
	Model 1 OR*	95% CI	Model 2 OR*	95% CI	Model 1 OR*	95% CI	Model 2 OR*	95% CI
bw/ga (continuous)	1.00	0.98, 1.01	1.00	0.98, 1.02	1.00	0.99, 1.01	1.00	0.98, 1.01
bw/ga ≥85 vs. <85%ile	0.79	0.21, 2.95	0.74	0.18, 2.94	0.73	0.22, 2.35	0.64	0.19, 2.17
bw/ga ≥95 vs. <95%ile	†	†	†	†	†	†	†	†
wt/ht 4 months (continuous)	1.00	0.98, 1.02	1.00	0.98, 1.02	1.00	0.99, 1.02	1.00	0.99, 1.02
wt/ht 4 months ≥85 vs. <85%ile	0.54	0.16, 1.81	0.66	0.18, 2.41	0.63	0.24, 1.64	0.74	0.27, 2.04
wt/ht 4 months ≥95 vs/ <95%ile	1.41	0.33, 6.00	1.16	0.24, 5.55	0.84	0.21, 3.43	0.77	0.18, 3.37
wt/ht 12 months (continuous)	1.01	0.99, 1.02	1.00	0.98, 1.02	1.00	0.99, 1.01	1.00	0.99, 1.01
wt/ht 12 months ≥85 vs. <85%ile	1.64	0.67, 4.03	1.78	0.66, 4.76	1.48	0.67, 3.26	1.51	0.36, 3.44
wt/ht 12 months ≥95 vs/ <95%ile	1.65	0.61, 4.45	1.30	0.44, 3.83	0.43	0.13, 1.36	0.40	0.12, 1.32
ssBMI 4 years (continuous)	1.00	0.98, 1.01	1.00	0.98, 1.02	1.00	0.99, 1.01	1.00	0.99, 1.01
ssBMI 4 years ≥85 vs. <85%ile	1.46	0.56, 3.84	1.22	0.44, 3.42	1.27	0.56, 2.91	1.09	0.45, 2.60
ssBMI 4 years ≥95 vs/ <95%ile	1.17	0.29, 4.70	1.07	0.26, 4.43	1.18	0.37, 3.77	1.02	0.31, 3.37
BMI 20s (continuous)	1.08	0.96, 1.21	1.08	0.95, 1.22	1.01	0.91, 1.12	1.01	0.91, 1.12
BMI 20s ≥25 vs. <25kg/m^2	***3.78***	***1.59, 9.00***	***3.52***	***1.42, 8.75***	1.09	0.54, 2.23	1.00	0.48, 2.09
BMI 20s ≥30 vs. <30kg/m^2	1.16	0.22, 6.06	1.30	0.22, 7.72	1.60	0.43, 5.98	2.09	0.50, 8.68

BMI 30s (continuous)	**1.09**	**1.00, 1.18**	***1.11***	***1.01, 1.21***	0.99	0.91, 1.08	1.00	0.92, 1.10
BMI 30s ≥25 vs. <25kg/m^2	2.13	0.84, 5.39	**2.52**	**0.94, 6.75**	0.57	0.28, 1.14	0.63	0.30, 1.32
BMI 30s ≥30 vs. <30kg/m^2	1.98	0.75, 5.19	**2.49**	**0.87, 7.10**	1.38	0.56, 3.41	1.35	0.51, 3.57
current BMI (continuous)	1.06	0.98, 1.14	1.06	0.98, 1.15	0.99	0.93, 1.07	0.99	0.92, 1.07
current BMI ≥25 vs. <25kg/m^2	1.11	0.43, 2.85	1.14	0.42, 3.13	0.99	0.44, 2.21	0.96	0.41, 2.27
current BMI ≥30 vs. <30kg/m^2	***2.33***	***1.05, 5.19***	***2.42***	***1.04, 5.69***	0.90	0.43, 1,89	0.85	0.39, 1.86

Bw/ga: sex-specific birth weight for gestational age percentile; wt/ht: sex-specific height-for-age percentile; ssBMI: sex-specific BMI percentile; BMI: body mass index

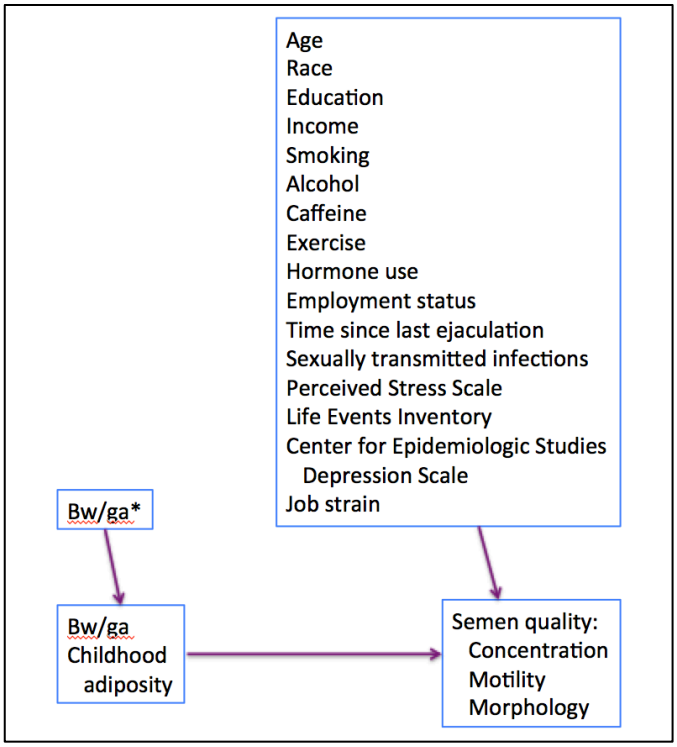
*Model 1 covariate-unadjusted; Model 2 adjusted for sex-specific birth weight for gestational age percentile, age, race, abstinence

† model failed to converge

bold italic: p-value <0.05; **bold:** p-value <0.10

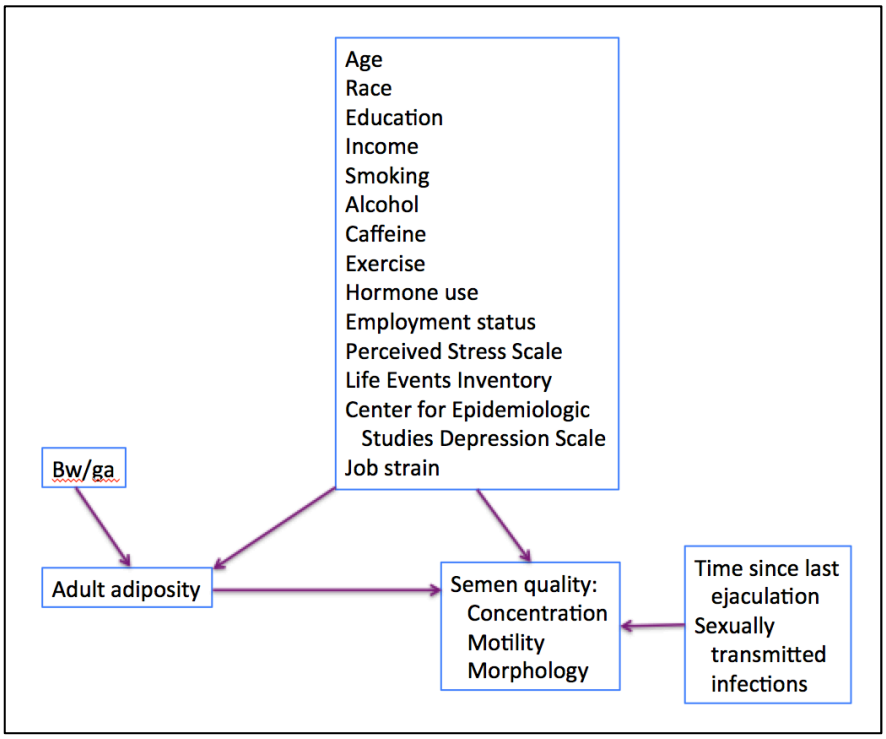
Supplemental Figure 2.1. Hypothesized relationships between adiposity, semen quality, and potential covariates.

A. Childhood

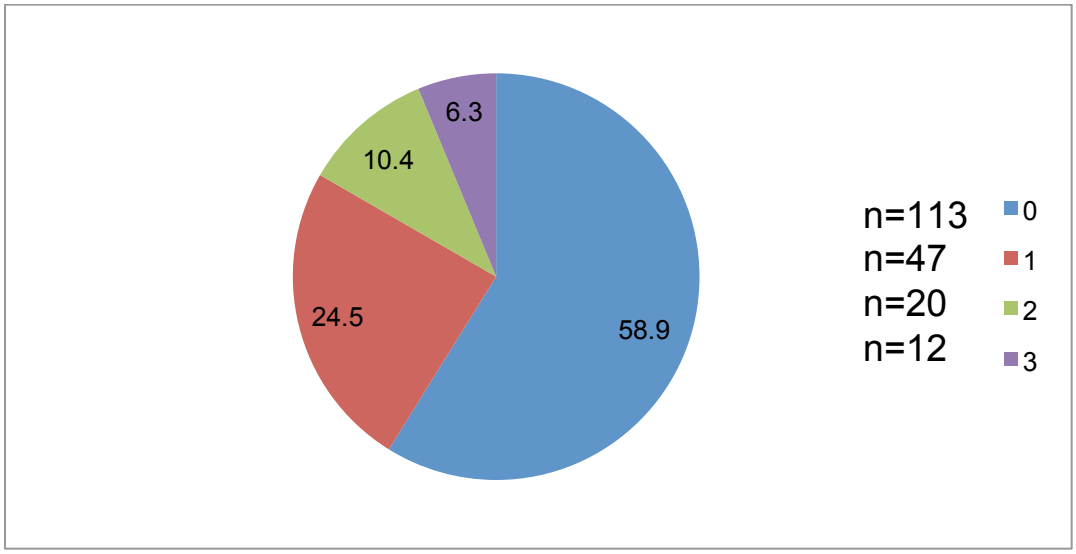


Bw/ga: sex-specific birth weight for gestational age percentile
*Bw/ga included in childhood adiposity analyses

B. Adulthood

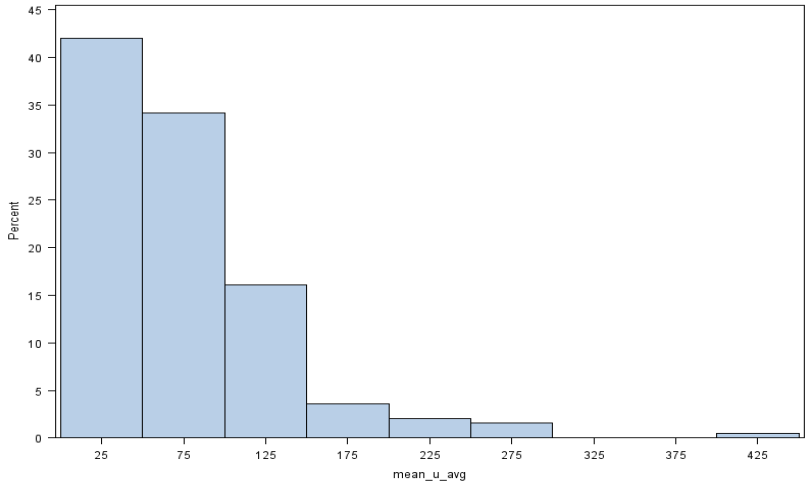


Supplemental Figure 2.2. Percentage of participants meeting 0, 1, 2, or 3 World Health Organization subfertility criteria.

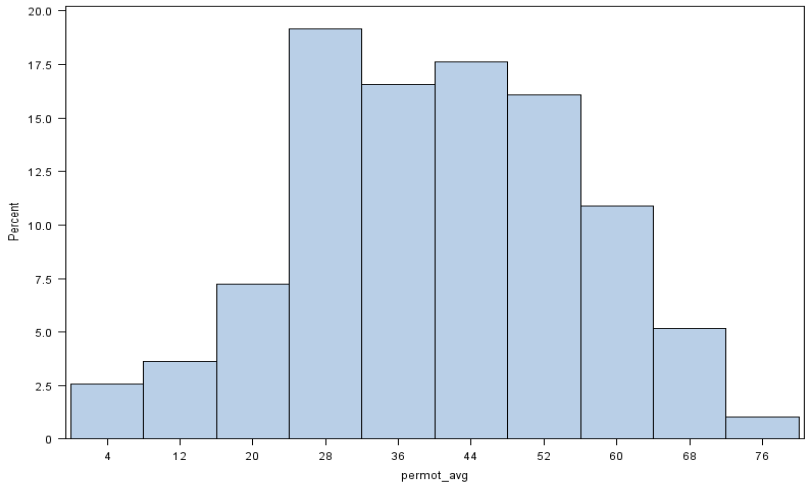


Supplemental Figure 2.3. Histograms of semen quality outcome measures.

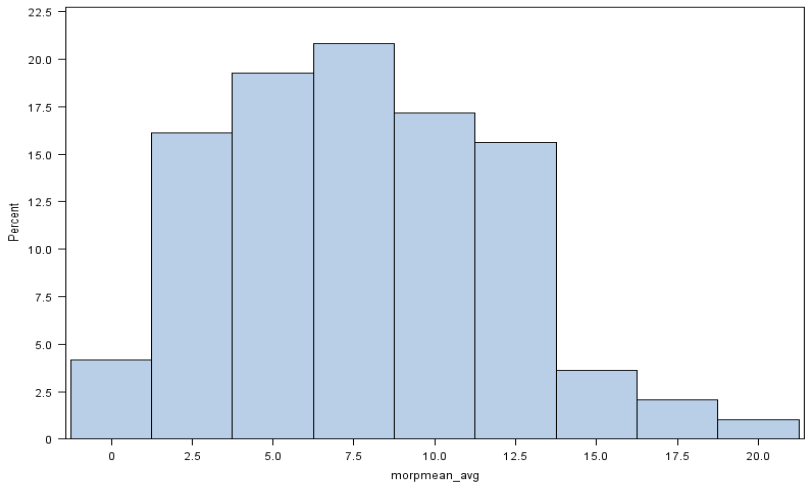
A. Sperm concentration



B. Percent progressive motility

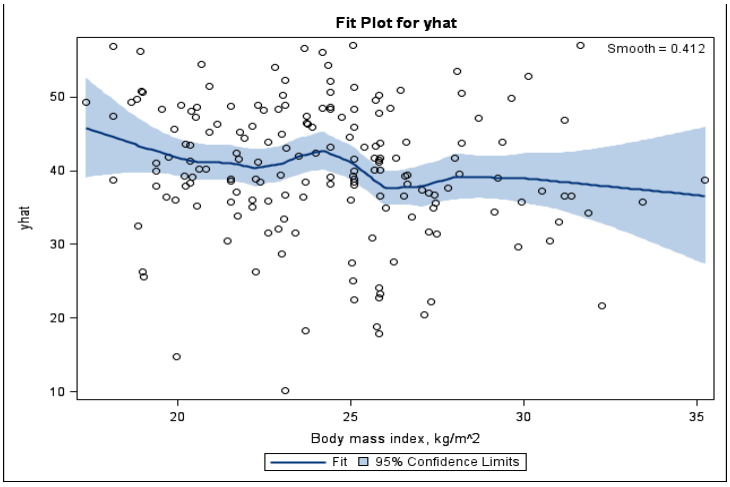


C. Percent normal morphology.

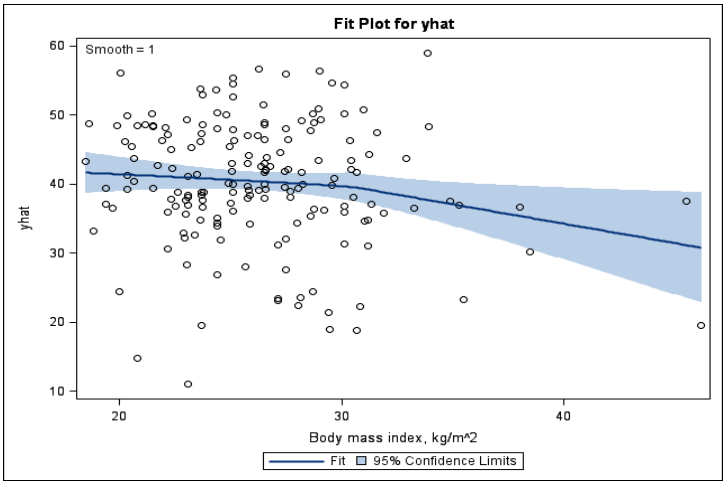


Supplemental Figure 2.4. Covariate-adjusted* loess smoothing curves of percent normal motility vs. adult BMI at three time points.

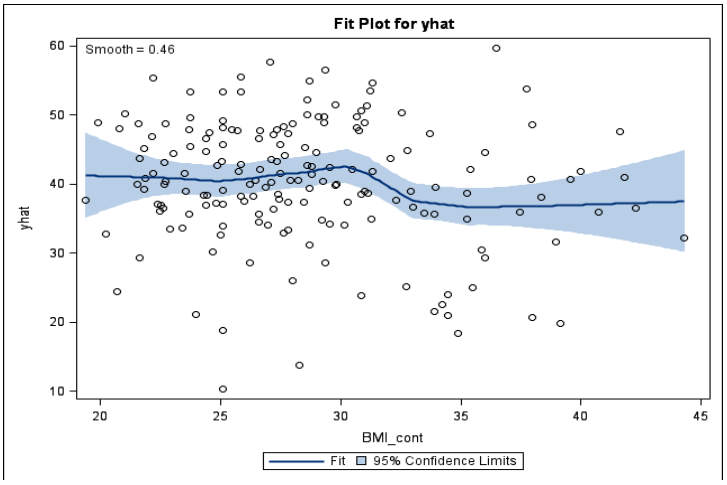
A. BMI in 20s



B. BMI in 30s



C. Current BMI

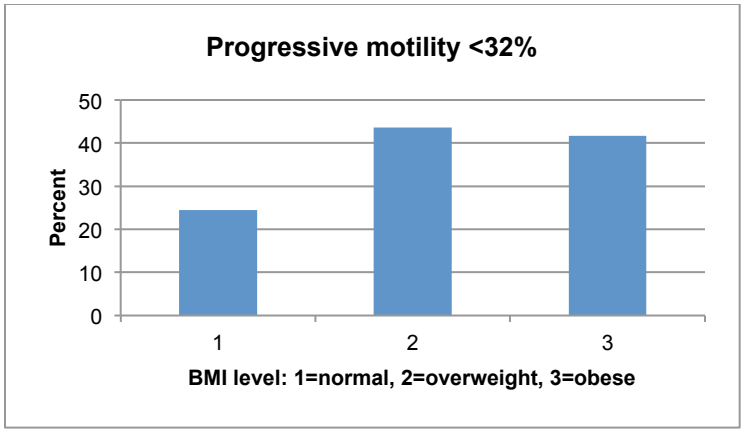


BMI: body mass index

*adjusted for birth weight for gestational age, current age, race, abstinence time, education, income, smoking, exercise, Perceived Stress Scale, Life Events Inventory, Center for Epidemiologic Studies Depression scale, employment status

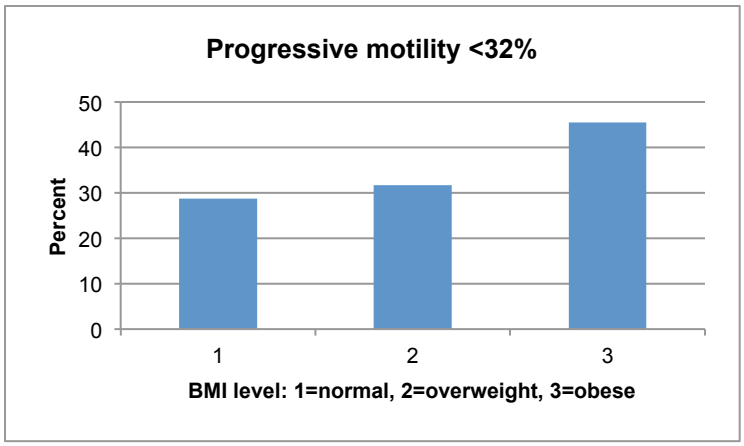
Supplemental Figure 2.5. Percentage of low progressive motility according to World Health Organization criteria by category of adult BMI at three time points.

A. BMI in 20s



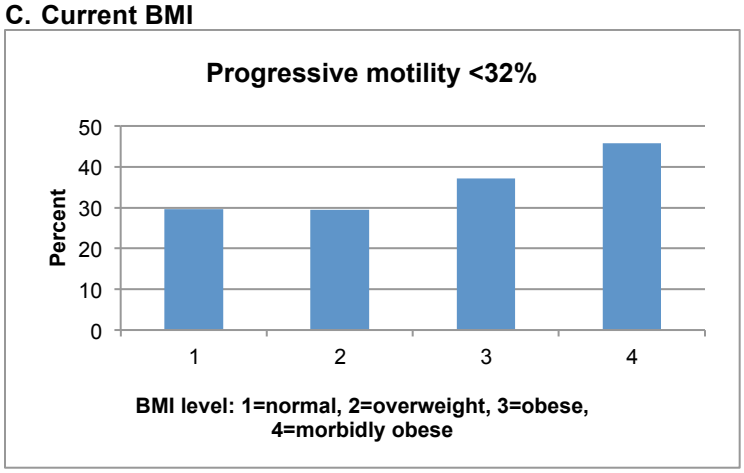
Chi-square Fisher's exact test p-value = 0.02
Two-sided exact Cochran-Armitage trend test p-value = 0.02

B. BMI in 30s



Chi-square Fisher's exact test p-value = 0.23
Two-sided exact Cochran-Armitage trend test p-value = 0.13

C. Current BMI



Chi-square Fisher's exact test p-value = 0.43
Two-sided exact Cochran-Armitage trend test p-value = 0.15

BMI: body mass index (kg/m^2)

CHAPTER 3. A PILOT STUDY OF THE RELATIONSHIP BETWEEN ALLOSTATIC LOAD AND SEMEN QUALITY IN AN URBAN FERTILITY CLINIC

3.1 ABSTRACT

Introduction: Psychosocial stress has been identified as a risk factor for poor semen quality, but most studies have examined only subjective or objective measures of recent stress and have consequently been limited in their ability to explore the biological relationship between stress and semen quality or the potential impact of cumulative stress over the life course on sperm production.

Objectives: We tested whether allostatic load, a biologically-based construct that theoretically reflects the effect of accumulated stress on the body's major regulatory systems, is associated with sperm concentration, percent progressive motility, and percent normal morphology in men recruited from an urban fertility clinic.

Methods: In this cross-sectional pilot study, 61 English-speaking, non-azoospermatic men, age 27 to 53 years, who were part of couples seeking fertility treatment at Columbia University's Center for Women's Reproductive Care completed a questionnaire, had weight and blood pressure measured, and provided blood and semen samples for analysis. An allostatic load scale was created using 10 biomarkers of cardiovascular, metabolic, hypothalamus-pituitary-adrenal axis, and immune system function. Regression was performed to assess associations between allostatic load and sperm concentration, percent progressive motility, and percent normal morphology, controlling for covariates. Partial Spearman correlations were used to examine associations between metabolic and non-metabolic subdomains of allostatic load and the three semen outcomes.

Results: Allostatic load was not associated with sperm concentration or percent normal morphology in our sample. Those with ≥1 allostatic load indicators had on average 17.71 (95% CI [6.03, 29.38]) points higher percent progressive motility than those with no allostatic load indicators, adjusting for covariates. This relationship appeared to be driven by the 6 subjects in the lowest allostatic load category, who differed from the rest on mean testosterone level and possibly other unmeasured variables. The metabolic subdomain of allostatic load was significantly positively correlated with both sperm

concentration and motility after adjustment for covariates, but there was no correlation with the non-metabolic subdomain.

Conclusion: This pilot study suggests that the relationship between cumulative stress, operationalized as allostatic load, and semen quality may differ from that of recent stress, and that metabolic function may be linked to sperm concentration and motility.

3.2 INTRODUCTION

Poor semen quality is the primary diagnostic criterion for male infertility. Its predictors are multiple and incompletely understood. Male factor infertility affects approximately 50 percent of the 7.3 million couples facing involuntary childlessness in the United States(7), contributing to the increasing use of costly assisted reproduction technologies(8). Psychosocial stress has been identified as a risk factor for poor semen quality (summarized in (173, 174)), but empirical results have been inconsistent. Most studies have examined only subjective or objective measures of stress and have consequently been limited in their ability to explore the biological relationship between stress and semen quality. They have also considered only recent stress exposure, and have not explored the potential impact of cumulative stress over the life course on sperm production. Repeated or chronic stress can become biologically "embedded," as it has been shown to structurally remodel parts of the brain, thereby altering production of stress hormones and cytokines that affect various physiological systems (reviewed in (175)). In this cross-sectional pilot study, we aimed to fill these gaps by testing whether allostatic load, a biologically-based construct that theoretically reflects the effect of accumulated stress on the body's major regulatory systems, is associated with sperm concentration, percent progressive motility, and percent normal morphology in men recruited from an urban fertility clinic.

Over the past three decades, a growing body of literature has suggested that acute or recent psychosocial stress may be inversely associated with sperm concentration, motility, and morphology, but study results have not been consistent, with many reporting no association(129, 151, 174, 176-204). A major source of that variability is the diversity of exposure measures used to capture the construct of stress. Many of these studies did not measure stress at all, but instead measured anxiety or depression using tools such as the Spielberger State-Trait Anxiety Inventory(179, 186, 196, 202), Zung's Anxiety Scale Inventory(204), and the Hospital Anxiety Depression Score(177). Others measured subjective psychosocial stress using stand-alone questions(129, 183, 195) or validated scales such as the Perceived Stress Scale(151, 192), the General Health Questionnaire(190), the Life Stress Questionnaire(184), or the Copenhagen Psychosocial Questionnaire(174). Job-related stress was captured through direct questions(178, 183) or validated scales such as the Job Content Questionnaire(151, 182, 189), the Subjective Work Characteristics Questionnaire(192), or the

Fragebogen zur Abschätzung des Psychosomatischen Krankheitsgeschehens(191). To assess objective stress, researchers had subjects tally recent acutely stressful life events(151, 182, 185, 204) or designed studies around specific stressful events, including medical exams(181, 194, 198), war(176, 203), or an earthquake(205). To measure stress specific to infertility, one group created an Infertility Distress Scale(199, 200), another used the Fertility Problem Inventory(197), while still others captured the stress of providing a semen sample for *in vitro* fertilization by comparing results of a previous diagnostic semen analysis to an analysis done on the day of the female partner's egg retrieval(180, 187, 188, 193, 201).

In addition to the various means of operationalizing stress used in these studies, the lack of consistency among their results may be attributable to several other factors, including a wide range of sample sizes and study populations, and inconsistent covariate control (Supplemental Table 3.1). Sample sizes ranged from 15 (in a study of medical students before and during exams)(194) to 10,782 (in a study of Lebanese men during and after the civil war)(176). Some studies included only men recruited from fertility clinics or andrology labs who were part of infertile couples(176-180, 183, 186-188, 191-193, 197, 199-201, 203-205), others sampled only men known to be fertile(129, 185), while still others sampled men from the general population(151, 182, 184, 195, 196). Most studies included men whose ages spanned the childbearing years, while others focused on exclusively young men such as military recruits(174), medical students(181, 194, 198), and first-time pregnancy planners(189, 190). The divergent sampling pools of these studies limit not only the generalizability of their results, but their ability to be compared to one another. Finally, fewer than half of the 33 analyses we reviewed adjusted for any covariates, and only 11 adjusted for both age and duration of abstinence prior to semen collection, one of several proposed criteria for high-quality semen studies(58).

All of these prior studies only consider the role of recent stress, most often in or around the 74-day period of spermatogenesis(41), and do not account for the potential of cumulative stress over the life course to affect regulatory systems in the body that may support healthy sperm production, including cardiovascular function, metabolism, hypothalamus-pituitary-adrenal (HPA) axis activity, and inflammation. Persistent exposure to stress may compromise the ability of these systems to appropriately respond to or recover from a stressful experience and regain homeostasis. This maladaptation causes wear-and-tear on the body known as allostatic load(43), based on the term "allostasis," which Sterling and

Eyer coined to describe this process of re-equilibration(206). Even if two men experience similar stressful events, their physiologic responses—how they embody that stress--may differ according to how they perceive it as well as how much allostatic load they have accumulated over their life course, both of which are influenced by prior stress exposure.

The concept of an allostatic load scale to capture the degree of homeostatic dysregulation was pioneered by Seeman et al. in their analysis of the MacArthur studies of successful aging, in which they created a 0-10 point scale to measure levels of allostatic load using parameters of physiologic activity across a range of domains associated with disease risk(44). Their scale included seven biomarkers (total cholesterol, high density lipoprotein (HDL), cortisol, epinephrine, norpeinephrine, glycosylated hemoglobin (HbA1c), and dehydroepiandrosterone sulfate (DHEA-S)) and three anthropometric measures (systolic and diastolic blood pressure, and waist-hip ratio). Each variable was dichotomized and one point was given to those in the highest risk quartile according to its distribution in the study sample. In a subsequent paper, Seeman et al. suggested adding C-reactive protein (CRP) and interleukin-6 (IL-6) to the scale in order to capture disturbance of the inflammatory response, an important adaptive process(207).

In the cardiovascular literature, there has been debate as to whether or not the construct provides additional information beyond the effect of metabolic syndrome alone, as several of the diagnostic criteria of metabolic syndrome such as abdominal obesity, low HDL cholesterol, and elevated triglycerides, blood pressure, and blood sugar are frequently included in allostatic load scales(44, 208-210). There is some evidence that metabolic syndrome may be associated with male infertility, as well(211, 212), potentially prompting a similar concern about hypothesizing an association between allostatic load and poor semen quality. Furthermore, it is possible that while some semen parameters may be more strongly affected by increased allostatic load in systems affiliated with metabolism, others may be more strongly affected by increased allostatic load in regulatory systems classified as non-metabolic, such as inflammation. Indeed, studies have shown elevated seminal plasma levels of inflammatory cytokines such as IL-6 to be associated with male infertility(213, 214).

We hypothesized that increased total allostatic load would be associated with lower sperm concentration, percent progressive motility, and percent normal morphology, and that the metabolic and

non-metabolic subdomains of the allostatic load scale would have different effects on the three semen parameters. Our purpose was to increase understanding of the biological mechanisms by which stress influences the male reproductive system and suggest potential avenues for clinical management of men at risk of or diagnosed with poor semen quality.

3.3 METHODS

3.3.1 Participants

The pilot study sample comprised 61 non-azoospermatic men (i.e., not lacking sperm in their semen), mean age 38.9 years, who were of unknown fertility status but were part of couples seeking fertility treatment at Columbia University's Center for Women's Reproductive Care (CWRC) in midtown Manhattan. Because of budget restrictions, sampling was restricted to English speakers. Participants were required not to have previously undergone semen analysis and to be providing both semen and blood samples on the day of the visit. Basic demographic features of the study population are presented in Table 3.1.

3.3.2 Procedures

Between May and December, 2014, eligible participants were sequentially recruited when they arrived at CWRC for their initial fertility workup. If they agreed to participate, they provided informed consent and filled out a 48-item self-administered written questionnaire that included self-reported height. Participants then provided a semen sample and, afterward, had nonfasting blood samples drawn as part of the standard infertility workup protocol; for research purposes, an additional two vials of blood were collected and weight and blood pressure were taken by trained clinic staff using a Detecto 439 Physician Beam Scale and Welch Allyn 300 Series Vital Signs Monitor, respectively. All non-fasting blood samples were collected between 9:40 am and 12:30 pm.

Semen samples were analyzed according to the 2010 World Health Organization (WHO) protocol(145) at the CWRC lab. Briefly, subjects produced semen samples by masturbation into sterile specimen cups (Fisher Scientific) in a private room after a recommended 2 to 5 days of abstinence. Each

sample was immediately transferred to the on-site diagnostic laboratory, where it was analyzed within 1 hour of collection by one of two trained andrologists. Upon liquefaction, volume was measured by weight, viscosity was determined, and pH was tested. After thorough mixing, one drop (3-5 µl) of semen was used to fill the two chambers of a calibrated MicroCell disposable counting chamber (Conception Technologies, San Diego, CA), and another drop was placed on a Fisherfrost Superfrost microscope slide (Fisher Scientific), tightly cover slipped to achieve a thin layer, and set aside for at least 15 minutes. Using a phase contrast microscope to observe the sample in one of the MicroCell chambers, sperm aggregation and agglutination were assessed and the concentration of non-spermatozoa ("round cells") was determined. With an eyepiece reticle fitted to the microscope and calibrated with a stage micrometer, 100 sperm were subsequently counted at x200 or x400 magnification and the number of grids containing the sperm were noted so that total sperm count and sperm concentration could be calculated. This procedure was repeated in the second MicroCell chamber. Motility was assessed by grading the movement of 100 spermatozoa in each of the two chambers as progressive, non-progressive, or immotile. For sperm count and motility, if there was <10% difference between the duplicate measures, the average measure was used. If there was >10% difference, the entire procedure was repeated. To assess morphology, the prepared slide was observed under oil at x1000 magnification and 100 sperm were appraised. Head, midpiece, and tail all had to be normal for a spermatozoon to be considered normal; any borderline forms were considered abnormal, according to Kruger's strict criteria(215).

Blood samples taken for the fertility workup were analyzed at CWRC for communicable diseases; blood samples taken for research purposes were refrigerated and transported to Columbia University Medical Center's Clinical and Translational Science Award (CTSA) Core Biomarkers Laboratory within 4 hours of collection, where they were aliquoted and frozen at -80°C prior to having allostatic load biomarkers and testosterone levels analyzed. The research protocol was approved by the Institutional Review Board of Columbia University.

3.3.3 Exposure measures

According to the methodology described by Seeman et al., an allostatic load scale was created by assigning one point to those in the highest-risk quartile of the study sample for each of 10 variables:

body mass index (BMI), systolic and diastolic blood pressure, total cholesterol, HDL cholesterol, albumin, CRP, HbA1c, DHEA-S, and IL-6(44) (see Supplemental Table 3.2 for assay descriptions). Table 3.2 shows the regulatory systems represented by the various indices along with each component's sample median, range, mean, standard deviation, and high-risk quartile cutoff point, as well as the clinical cut point above or below which is considered abnormal and the percent of subjects with values beyond the clinical cut point. Men who reported taking cholesterol- or blood pressure-lowering medication were classified as high risk for those measures. Allostatic load was modeled both as a count variable and dichotomously in order to describe the pattern of its association with the outcome measures. For purposes of sensitivity analyses, an alternate allostatic load scale was created using clinical rather than sample-based quartile cut points for the 10 components. Scales were also created that represented the metabolic (cardiovascular and metabolism) and non-metabolic (HPA axis and inflammation) subdomains of allostatic load.

3.3.4 Outcome measures

The three semen parameters—sperm concentration, percent progressive motility, and percent normal morphology--were modeled both as continuous variables and dichotomously using 2010 WHO reference values for poor semen quality: sperm concentration <15 million/mL, percent progressive motility <32%, percent normal morphology <4%(147).

3.3.5 Covariates

Information on behavioral, sociodemographic, medical, and psychosocial risk factors for infertility were assessed via written questionnaire. Covariates identified as potential confounders based on a review of the literature and a causal diagram (directed acyclic graph) included: age (continuous, years), race (white vs. other), US birth (Y/N), education (high school or some college, bachelor's degree, graduate level), income (<$100,000, $100,000-150,000, >$150,000), smoking status (current vs. not current), caffeine consumption (Y/N), alcohol consumption (Y/N), and exercise (any vs. none). Although abstinence (continuous, days), time from ejaculation to semen analysis (continuous, minutes), genital

surgery (Y/N), and diagnosis with a sexually transmitted infection (ever/never), do not meet the criteria for confounders, they were considered because of their potential contribution to the variance of the outcome measures.

Allostatic load theoretically represents cumulative stress over the life course, but some of its components may be influenced by recent stress, so we also considered the following measures of current anxiety, stress, and depression: anxiety related to fertility treatment (Y/N), operationalized as the question "Were you ever concerned about a possible problem with your fertility?"; the validated abbreviated 10-item Perceived Stress Scale (PSS) assessing feelings during the last month(152); a shortened form of the Life Events Inventory (LEI) assessing experiences during the last 3 months(153), which includes the top 10 stressors of men from an occupational sample(154); the 16-item Job Content Questionnaire (JCQ) assessing job requirements and satisfaction during the last 3 months(155); and the 20-item Center for Epidemiologic Studies Depression Scale (CES-D) assessing feelings and experiences during the last week(156) (Supplemental Figure 3.1). PSS, LEI, and CES-D were modeled continuously; a dichotomous job strain variable was created that categorized those above the sample median job demand score and below the sample median job control score as high strain. Because BMI is one of the items in the allostatic load scale, it was not considered as a covariate in the regression models.

3.3.6 Statistical analyses

Univariate analyses were performed to describe characteristics of the cohort and to assess the distributions of the three outcome variables--sperm concentration, percent progressive motility, and percent normal morphology.

In bivariate analyses, analysis of variance was used to compare means of the count/continuous exposure and outcome variables according to levels of potential categorical covariates. Chi-square tests were used to detect bivariate associations between categorical covariates. Nonparametric Spearman correlations were used to examine associations between potential continuous covariates as well as between continuous covariates and both exposure and outcome variables. Spearman correlations among the three semen parameters and among the 10 items in the allostatic load scale were also assessed.

We decided *a priori* to include age, time from ejaculation to semen analysis, and duration of ejaculatory abstinence in each of the final models. To select additional covariates for control in the regression models, separate simple linear regression models were run to examine the association between allostatic load score and each of the three outcome variables. Hypothesized confounders and predictors of the outcomes that changed the estimated regression coefficient of the predictor in the simple model by at least 50% of the standard error were included in the relevant covariate-adjusted models.

Linear regression models with allostatic load as the quantitative predictor for the three continuous semen outcomes were run with and without covariates. Logistic regression models with allostatic load as the quantitative predictor for the three dichotomous semen outcome variables (based on WHO reference levels) were run with and without the same covariates as in the corresponding linear models. Boxplots of allostatic load score vs. each of the continuous semen parameters were created to visualize the direction and shape of the relationships. Bar graphs were created to show the relative percentage of subjects with semen variables below the WHO reference levels for each allostatic load score.

In post-hoc analyses, where there appeared to be potential threshold effects, dichotomous allostatic load variables were created at the points suggested by the boxplots. To account for possible unequal variance in outcome within the allostatic load groups, we used linear models with heteroscedasticity-consistent standard errors(216, 217) to assess associations between binary allostatic load variables and the three semen outcomes, with and without covariates. Because of concern that recent psychosocial stress might contribute to allostatic load, in which case including stress variables in our models could bias the estimates of the associations through overadjustment(150), we compared results of the final covariate-adjusted models that had sperm concentration as the outcome to models without job strain (the only stress variable that met the criteria for inclusion in any of the final models). Additionally, when it appeared that those with an allostatic load score = 0 had substantially lower percent progressive motility than the rest, we compared results of the final covariate-adjusted models that had motility as the outcome to models that included only those with allostatic load ≥1.

In alternative analyses, all regression models were repeated using allostatic load scores based on clinical cut points.

Because the total allostatic load and its metabolic and non-metabolic subdomains contained different numbers of items, in secondary analyses we used covariate-adjusted partial Spearman correlations to describe the strength and direction of the associations among the three configurations of allostatic load and the three semen outcomes. We also examined whether each of the ten items of the total allostatic load scale, measured continuously, was correlated with the outcome measures. All analyses were performed in SAS 9.3 (SAS Institute, Cary, NC).

3.4 RESULTS

Allostatic load score, calculated using the sample cut points, was right-skewed, with a median/mode of 2 and a range of 0-7 out of 10 (using clinical cut points, median/mode = 1 and range = 0-5 out of 10). There was weak to moderate correlation among the allostatic load items within the metabolic and non-metabolic subdomains (Supplemental Table 3.3). No extreme values were apparent in univariate analysis of the three outcome measures and upon visual inspection of histograms it was determined that they did not require transformation to meet the normality assumption for linear regression (Supplemental Figure 3.2). Mean sperm concentration was 47.4×10^6/mL (standard deviation (SD) 29.0); mean percent progressive motility was 41.2 (SD 14.3); mean percent normal morphology was 2.3 (SD 1.7) (Table 3.1). The three semen parameters were significantly correlated with one another (range of correlation coefficients: 0.27-0.41) (Supplemental Table 3.4).

Allostatic load was not associated with any of the current stress or depression scales in our model (Supplemental Table 3.5).

In our sample, 13.1, 26.2, and 75.4 percent of participants were below the 2010 WHO reference values for sperm concentration, progressive motility, and normal morphology, respectively (Supplemental Figure 3.3). Only 18.0 percent of our sample was above the reference values for all three parameters (Supplemental Figure 3.4). Histograms of the three semen outcomes appeared approximately normally distributed.

Boxplots of mean semen parameters for each allostatic load level suggested a potential threshold effect between clinic-based allostatic load score = 0 and ≥1 for concentration, between both sample- and clinic-based allostatic load score = 0 and ≥1 for progressive motility, and between sample-based allostatic

load score ≤4 and ≥5 for morphology (Figure 3.1; note: the top two levels of allostatic load were collapsed because of sparse data in the highest level).

3.4.1 Concentration

In linear regression analyses, sample-based allostatic load score, modeled as a count variable, was not associated with sperm concentration in our data, regardless of whether we used sample-derived or clinical cut points. When we tested the clinic-based dichotomous allostatic load variable 0 vs. ≥1 to explore the potential threshold seen in the boxplot, we found that the 49 subjects with clinic-based allostatic load ≥1 had on average 17.52 million more sperm/mL (95% CI [1.38, 36.41]) compared to the 12 subjects with allostatic load = 0 in regression analysis adjusting for covariates. When we did not adjust for job strain, the association was stronger (b_{adj} = 21.14, 95% CI [3.12, 39.16]). This suggests that job strain may contribute to allostatic load, as adjusting for it weakens the positive relationship between allostatic load and sperm concentration. In logistic regression analyses, there was no association between either sample-based or clinic-based allostatic load score and low sperm concentration according to the WHO reference value (Table 3.3).

3.4.2 Motility

Modeled as a count variable, sample-based allostatic load was not significantly associated with motility in linear regression analyses. When the sample-based allostatic load score was modeled dichotomously, the adjusted percent progressive motility was on average 17.71 (95% CI [6.03, 29.38]) points higher among the 55 subjects with an allostatic load score ≥1 compared to the 6 subjects with an allostatic load score of 0. When the clinic-based allostatic load count variable was used, there was a statistically significant positive relationship with motility (b_{adj} = 3.57, 95% CI [0.66, 6.48]). When the 12 subjects with the lowest clinic-derived allostatic load score were removed, the association again disappeared (b_{adj} = 1.60, 95% CI [-1.56, 4.75]). When this model was run using the clinic-based dichotomous allostatic load variable, the 49 subjects with an allostatic load score ≥1 had on average 15.79 percentage points (95% CI [6.72, 24.85]) higher progressive motility compared to the 12 subjects

with an allostatic load score of 0 in adjusted analyses. Using logistic regression, there was no association between sample-based or clinic-based allostatic load score and low percent progressive motility according to the WHO reference value (Table 3.3).

3.4.3 Morphology

In unadjusted and adjusted linear models, we found no association between either sample-based or clinic-based allostatic load score, modeled as a count variable, and sperm morphology. Using a sample-based dichotomous allostatic load variable suggested by the boxplot, the adjusted percent normal morphology was on average 1.10 (95% CI [0.12, 2.08]) points lower among the 10 subjects with an allostatic load score ≥5 compared to the 51 subjects with an allostatic load score ≤4. In logistic regression analyses, there was no association between sample-based or clinic-based allostatic load score and low percent normal morphology according to the WHO reference value (Table 3.3).

Partial Spearman correlations were run between each of the semen outcomes and the total allostatic load score, its metabolic and non-metabolic subdomains, and the 10 individual components of allostatic load. While none of the outcomes was correlated with the sample-based total allostatic load score, sperm concentration and progressive motility were positively correlated with the metabolic subdomain and negatively but not statistically significantly correlated with the non-metabolic subdomain. Systolic blood pressure, HbA1c, and serum albumin were also positively correlated with sperm concentration, while BMI was positively but not significantly correlated with percent progressive motility. Percent normal morphology was not correlated with any of the allostatic load measures (Table 3.4).

3.5 DISCUSSION

The results of our analyses do not support our hypothesis of an inverse relationship between allostatic load and sperm concentration, motility, and morphology, as has generally been observed in prior research on current stress and semen quality. Instead we found no association between allostatic load and concentration or morphology and, surprisingly, a positive association between allostatic load and

motility. While it is possible that our results reflect differential effects of current and cumulative stress on semen parameters, there are several other potential explanations for these discrepancies.

While the mean percent progressive motility in our sample was comparable to that in other infertility cohorts of similarly aged men, the mean sperm concentration was slightly lower(218). Percent normal morphology in our sample was also lower than that in other published studies conducted in infertility clinics that used strict criteria(219-221). These differences in distribution, coupled with our small sample size, may have diminished our ability to detect an association between allostatic load and sperm concentration and morphology.

Our results may also have been influenced by the distribution of allostatic load in our cohort. In most domains, our subjects were generally healthy, as few exceeded the clinical high-risk cutoffs for measures of cardiovascular, HPA axis, and inflammatory system function; however, between 20% and 36% met each of the clinical high-risk criteria for impaired metabolic function (Table 3.2). Our cohort was of higher socioeconomic status than the general US population, as evidenced by the fact that all of our participants had health insurance, nearly three-quarters had at least a bachelor's degree, and only 2 earned less than $50,000/year (Table 3.1). They were also likely more advantaged than men included in studies that sampled from fertility clinics in countries where assisted reproduction is provided as part of national health systems and is not only available to those who can pay out of pocket or have health insurance that covers the procedure. Our participants' overall health and lack of adversity is reflected in the low maximum allostatic load scores in our sample: 7 out of 10 and 5 out of 10 using sample-derived and clinical cut points, respectively. After 7 years of observing their cohort of substantially older US adults (age 70-79 at recruitment), Seeman et al. observed a nonlinear relationship between allostatic load and mortality as well as cognitive and physical functioning, with a notably increased effect in the top allostatic load category (a score of 7-10 out of 10)(210). If the effect of allostatic load on semen quality were similarly concentrated at the high end of the allostatic load spectrum, our study would not have been able to capture it.

Another possible explanation of the discrepancy between our results and others' is that our allostatic load scale lacked measures of epinephrine, norepinephrine, and cortisol, all of which increase in the context of experienced or perceived stress (reviewed in (222, 223)). These key indicators of

sympathetic nervous system and HPA axis activity may indicate the biological mechanism that explains prior published findings of inverse associations between stress and semen quality. Furthermore, the serum biomarkers we did measure may not accurately reflect the chemical milieu in the testes, where sperm are produced. For example, concentrations of anti-Müllerian hormone, which is released by Sertoli cells in the testes, differ when measured in blood vs. semen; levels in seminal plasma have been found to be positively associated with sperm concentration, while there is no association with levels in serum(224). Similarly, levels of fatty acids, which have been shown to be associated with sperm morphology(225), differ between serum and seminal plasma(226, 227). It may be necessary to measure biomarkers of allostatic load in seminal plasma to accurately gauge the effect of homeostatic dysregulation on semen quality. As in all observational studies, there is also the possibility of residual confounding by unmeasured covariates.

Our finding of a positive association between allostatic load and progressive motility is driven by the 6 subjects with no allostatic load risk factors, who had significantly lower percent motile sperm than those in all other categories. Those subjects did not differ from the rest of the cohort on any of the hypothesized potential covariates; they did, however, have higher mean testosterone than the rest (523.33 ± 192.57 ng/dL vs. 391.82 ± 142.80 ng/dL) (Supplemental Table 3.6). Higher testosterone was significantly associated with lower progressive motility in the overall cohort, both including and excluding the 6 subjects. This is in contrast to published observational studies that have found either a positive association(177, 228, 229) or no association(80, 230-232) between testosterone and sperm motility. When testosterone was added to the adjusted model of the relationship between allostatic load and motility, the result was still significantly positive, but the estimated beta was slightly closer to the null (b_{adj} =13.62; 95% CI, 1.39, 25.86 vs. 17.71; 95% CI, 6.03, 29.38), suggesting potential partial mediation by testosterone. The remaining strength of the testosterone-adjusted relationship indicates either the presence of an unmeasured variable that distinguishes those in the lowest allostatic load category from the rest or is simply a spurious result due to our small sample size.

It is notable that the partial correlation coefficients of the metabolic and non-metabolic subdomains of allostatic load are in opposite directions in relation to sperm concentration and progressive motility, suggesting that distinct biological pathways may influence these aspects of sperm production. It

is also notable that the individual allostatic load components that are most responsive to fluctuations in current conditions—systolic and diastolic blood pressure, HbA1c, and serum albumin—were most strongly and positively correlated with sperm concentration. The only component significantly associated with morphology was DHEA-S in men between ages 40 and 50, which suggests that as HPA axis activity decreases with age, effects on sperm morphology may become more pronounced (Table 3.4). When individual components of allostatic load that may be driving the total score (especially in a low-stress population) are responsive to recent stress, it begs the question of allostatic load's validity as a construct for measuring the impact of cumulative stress on the body.

In cases when biological processes and subsystems appear to work in sync to influence a particular health outcome, such as cardiovascular disease, associations between allostatic load and that health outcome may be stronger than those of individual markers, as seen in Seeman et al.'s analysis of the MacArthur Studies(44). In our study, by contrast, it appears that alterations in metabolic and non-metabolic (primarily inflammatory) systems' responses to stress may have opposing effects on semen quality, which are obscured when all components of the scale are combined, throwing into question the value of allostatic load as a unified construct. It is also possible that allostatic load reflects cumulative stress most reliably in older or highly stressed populations in which there are more subjects with total scores at the high end of the scale.

3.5.1 Limitations

There are several limitations to this pilot study. First, its cross-sectional design does not allow for the firm establishment of temporality between allostatic load and semen quality. Because allostatic load is a construct designed to capture cumulative stress over the life course and contains measures that reflect long-term biological processes, however, it theoretically should reflect embodied stress prior to the period of spermatogenesis. Second, because of the infeasibility of collecting first morning urine or a 12-hour urine sample in this pilot study, norepinephrine, epinephrine, and cortisol were not included in the allostatic load scale (a random urine sample would not yield reliable measures of these hormones, which vary diurnally(233)). Third, because subjects were recruited when they arrived at the clinic, they were not instructed to fast prior to having their cholesterol measured; however, fasting does not substantially affect

total and HDL cholesterol measures according to a large Canadian study(234). Fourth, because of restrictions on nursing staff availability, only a single blood pressure measure was taken. Because initial blood pressure readings are generally elevated, especially in clinical settings(235), it is possible that there could have been nondifferential misclassification of systolic and diastolic blood pressure measurements, but any resulting bias would be small and toward the null. Subjects were also weighed rather than having their waists and hips measured, therefore BMI was used as a component of the allostatic load score rather than waist-hip ratio, the preferred anthropometric measure of central adiposity. Fifth, although it is considered optimal for two semen samples to be obtained and the results averaged, CWRC subjects only provided a single sample. Studies conducted in both Norway and China have concluded that for epidemiologic research, as contrasted to clinical diagnosis, a single sample is a sufficiently accurate indicator of semen quality(236, 237). Sixth, the small sample size reduced the likelihood of finding statistically significant results and prohibited factor analyses of the allostatic load scale. Factor analysis would have been useful in determining whether the division into metabolic and nonmetabolic subdomains best reflected the underlying structure of the data. Performing multiple comparisons in such a small cohort is unfortunately unavoidable; this study was intended to provide impetus for a larger study in the future. Finally, as is common in semen quality research, the generalizability of the results is restricted because participants were recruited from a fertility clinic.

An additional limitation that applies not only to this study but to all studies of allostatic load is the inability to fully distinguish the biological effects of cumulative stress from current stress. Several of the measures commonly included in allostatic load, such as systolic and diastolic blood pressure and HbA1c, can vary substantially in response to current or recent conditions, and transient high levels can increase allostatic load scores. In the case of a health outcome that takes years or decades to develop, it may still be convincingly argued that an association with allostatic load may be attributable to cumulative stress. In the case of a health outcome such as spermatogenesis, which takes place within a relatively short time period, such a conclusion is less certain.

3.5.2 Strengths

The main strengths of this study are the high-quality exposure and outcome measures. Blood samples were all taken at the same time of day and were analyzed at an NIH-funded CTSA research laboratory. The seven biomarker assays have excellent inter- and intra-assay precision measures (Supplemental Table 3.2), and all of the blood samples were run simultaneously, averting any batch effect. The semen was collected and processed according to a validated protocol by experienced clinical and laboratory personnel.

3.5.3 Conclusion

Allostatic load was not associated with sperm concentration or percent normal morphology in our diverse sample of men from an urban fertility clinic. Those with ≥1 allostatic load indicators had on average 17.71 (95% CI [6.03, 29.38]) points higher percent progressive motility than those with no allostatic load indicators, adjusting for covariates. This relationship appeared to be driven by the 6 subjects in the lowest allostatic load category who differed from the rest on mean testosterone level and possibly other unmeasured variables. The metabolic subdomain of allostatic load was significantly positively correlated with both sperm concentration and motility in adjusted analysis, and there was a negative but not statistically significant correlation with concentration. This pilot study suggests that the relationship between cumulative stress, operationalized as allostatic load, and semen quality may differ from that of recent stress, and that metabolic function may be linked to sperm concentration and motility. It also highlights the caveat that using a total allostatic load score to measure the impact of cumulative stress on a health outcome may mask associations with particular subdomains or even individual biomarkers that can provide information useful for identifying biological mechanisms. Future studies of allostatic load and semen quality that collect additional markers of HPA axis and sympathetic nervous system activity and that measure biomarkers in seminal plasma are warranted.

Table 3.1. Characteristics of the Center for Women's Reproductive Care sample (n=61).

	mean (SD)	median (range)
Age	38.9 (5.9)	37.8 (27-53)
BMI (continuous)	28.2 (5.1)	26.5 (20.1-44.3)
Concentration (10^6/mL)	47.4 (29.0)	43.7 (0.2-132.1)
% Progressive motility	41.2 (14.3)	40 (8-69)
% Normal morphology	2.3 (1.7)	2 (0-6)
Testosterone (ng/dL)	404.8 (151.7)	358 (111-843)
	n (percent)	
US birth		
Yes	36 (59.0)	
No	25 (41.0)	
Race		
White	37 (60.7)	
Black	4 (6.6)	
Hispanic	15 (24.6)	
Other	5 (8.2)	
Education		
<Bachelors degree	17 (27.9)	
Bachelors degree	19 (31.2)	
>Bachelors degree	25 (41.0)	
Income		
<$100,000	22 (36.7)	
$100-150,000	11 (18.3)	
>$150,000	27 (45.0)	
BMI		
<25	17 (27.9)	
≥25 to <30	26 (42.6)	
≥30 to <35	10 (16.4)	
≥35	8 (13.1)	
Current smoking		
Yes	4 (6.6)	
No	57 (93.4)	
Exercise		
None	6 (10.0)	
>0 to <4 hours/week	17 (28.3)	
4 to 8 hours/week	20 (33.3)	
>8 hours/week	17 (28.3)	
Job strain		
Low	43 (78.2)	
High	12 (21.8)	
Alcohol		
None	8 (13.1)	
Any	53 (86.9)	

Table 3.2. Allostatic load indicies by regulatory system, CWRC sample (n=61).

	Median	Range	Inter-quartile range	Mean	Standard deviation	High-risk quartile	Sample cutoff	Clinical cutoff
Cardiovascular								
Systolic blood pressure (mmHg)	122.5	106-158	116.5-131.5	124	11.2	highest	≥132	≥140
Diastolic blood pressure (mmHg)	77	58-106	71.0-82.5	77.9	9.9	highest	≥83	≥90
Metabolism								
Glycosylated hemoglobin (%)	5.4	4.8-6.20	5.2-5.6	5.4	0.3	highest	≥5.6	>5.6
Total cholesterol (mg/dL)	181	91-279	165-202	182.4	33.1	highest	≥203	≥200
HDL cholesterol (mg/dL)	47	28-78	37-55	47.6	12.8	lowest	≤37	<40
Body mass index (kg/m²)	26.5	20.1-44.3	24.6-30.6	28.2	5.1	highest	>30.6	≥30
HPA axis								
Dehydroepiandrosterone sulfate (µg/dL), age 30-39	225	61.1-520.0	180-308	243.1	110.8	lowest	≤180	<120
Dehydroepiandrosterone sulfate (µg/dL), age 40-50	206	50.4-448.0	153-319	219.6	97.4	lowest	≤153	<95
Inflammation								
C-reactive protein (mg/dL)	0.7	<0.3-10.1	0.3-1.7	1.4	1.9	highest	≥1.66	>3
Interleukin-6 (pg/mL)	0.2	<0.2-4.8	0.2-0.2	0.7	1.1	highest	≥2.0	>4.3
Serum albumin (g/dL)	4.4	3.6-5.1	4.2-4.6	4.4	0.3	lowest	≤4.2	<3.5

CWRC: Center for Women's Reproductive Care; HDL: high-density lipoprotein

Table 3.3. Regression of continuous semen outcomes on various parameterizations of allostatic load.

A. Sample high-risk cut points

Allostatic load parameterization	Covariate unadjusted		Covariate adjusted		Covariate unadjusted		Covariate adjusted	
	b	95% CI	b	95% CI	OR	95% CI	OR	95% CI
	Continuous concentration ($x10^6$/mL)†				Dichotomous concentration (<$15x10^6$/mL)†			
Count (0-7)	-1.03	-5.40, 3.34	0.27	-4.19, 4.72	1.22	0.81, 1.85	1.04	0.52, 2.08
Count (0-7, without adjusting for job strain)			1.12	-3.54, 5.78				
Dichotomous (≥1 vs. 0)	6.84	-16.62, 3.30	6.77	-20.46, 34.00				
Dichotomous (≥1 vs. 0, without adjusting for job strain)			11.17	-15.37, 37.71				
	Continuous progressive motility (%)††				Dichotomous progressive motility (<32%)††			
Count (0-7)	1.00	-1.15, 3.14	1.90	-0.44, 4.24	0.99	0.71, 1.38	0.88	0.60, 1.30
Count (1-7)			0.43	-2.40, 3.27				
Dichotomous (≥1 vs. 0)	***15.91***	***6.27, 25.56***	***17.71***	***6.03, 29.38***				
Dichotomous (≥1 vs. 0, additionally adjusted for testosterone)			***13.62***	***1.95, 25.30***				
	Continuous normal morphology (%)†††				Dichotomous normal morphology (<4%)†††			
Count (0-7)	-0.002	-0.26, 0.25	-0.07	-0.31, 0.18	1.09	0.77, 1.55	1.22	0.80, 1.85
Dichotomous (≥5 vs. ≤4)	-0.67	-1.61, 0.26	***-1.10***	***-2.08, -0.12***				

bold ital: p-value <0.05
† adjusted for US birth, age, abstinence, time to analysis, exercise, job strain
†† adjusted for US birth, age, abstinence, time to analysis, exercise
††† adjusted for US birth, age, abstinence, time to analysis, income

B. Clinical high-risk cut points

Allostatic load parameterization	Covariate unadjusted		Covariate adjusted		Covariate unadjusted		Covariate adjusted	
	b	95% CI	b	95% CI	OR	95% CI	OR	95% CI
	Continuous concentration (x10^6/mL)†				Dichotomous concentration (<15x10^6/mL)†			
Count (0-7)	0.22	-5.17, 5.62	3.66	-2.04, 9.39	1.08	0.64, 1.83	0.63	0.24, 1.66
Count (0-7, without adjusting for job strain)			3.27	-2.63, 9.17				
Dichotomous (≥1 vs. 0)	12.6	-4.21, 29.41	***17.52***	***1.38, 36.41***				
Dichotomous (≥1 vs. 0, without adjusting for job strain)			***21.14***	***3.12, 39.16***				
	Continuous progressive motility (%)††				Dichotomous progressive motility (<32%)††			
Count (0-7)	2.42	-0.17, 5.00	***3.57***	***0.66, 6.48***	0.80	0.51, 1.24	0.68	0.39, 1.17
Count (1-7)			1.60	-1.56, 4.75				
Dichotomous (≥1 vs. 0)	***13.61***	***4.79, 22.42***	***15.79***	***6.72, 24.85***				
Dichotomous (≥1 vs. 0, additionally adjusted for testosterone)			***12.53***	***3.37, 21.69***				
	Continuous normal morphology (%)†††				Dichotomous normal morphology (<4%)†††			
Count (0-7)	-0.08	-0.40, 0.23	-0.10	-0.41, 0.22	1.16	0.75, 1.79	1.39	0.79, 2.44

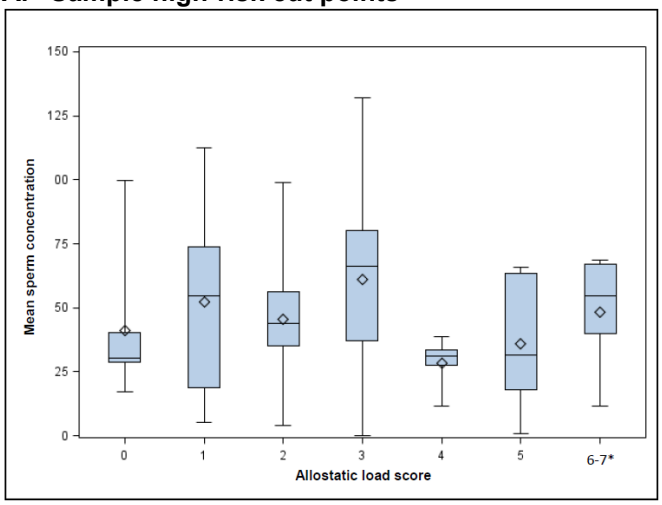
bold ital: p-value <0.05
† adjusted for US birth, age, abstinence, time to analysis, exercise, job strain
†† adjusted for US birth, age, abstinence, time to analysis, exercise
††† adjusted for US birth, age, abstinence, time to analysis, income

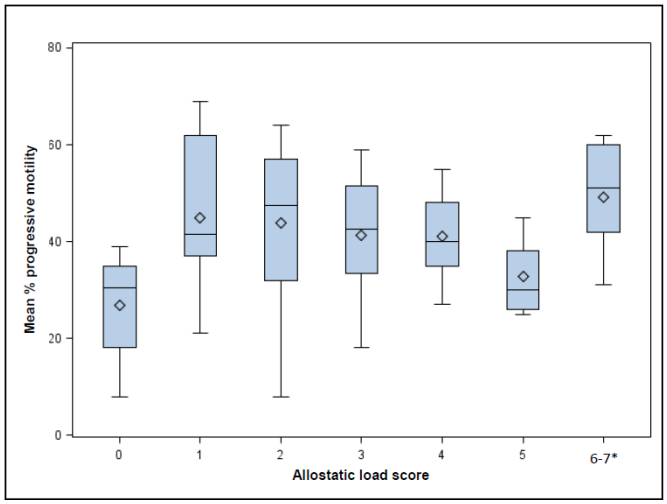
Table 3.4. Partial Spearman correlations among semen parameters and allostatic load: total, subdomains, and components.

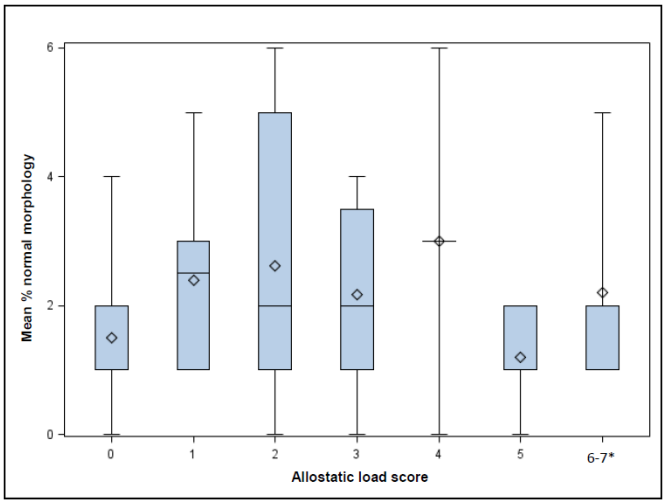
	Sperm concentration†			% Progressive motility††			% Normal morphology†††		
	n	coefficient	95% CI	n	coefficient	95% CI	n	coefficient	95% CI
Allostatic load, sample cut points									
Total	55	0.05	-0.24, 0.33	60	0.16	-0.11, 0.41	60	-0.02	-0.29, 0.24
Metabolic subdomain	55	**0.30**	**0.02, 0.54**	60	**0.32**	**0.06, 0.54**	60	0.03	-0.24, 0.29
Non-metabolic subdomain	55	-0.24	-0.49, 0.04	60	-0.06	-0.32, 0.21	60	-0.07	-0.32, 0.20
Allostatic load, clinical cut points									
Total	55	0.22	-0.06, 0.47	60	**0.32**	**0.05, 0.53**	60	0.01	-0.25, 0.28
Metabolic subdomain	55	0.23	-0.05, 0.48	60	**0.31**	**0.05, 0.53**	60	0.02	-0.24, 0.29
Non-metabolic subdomain	55	0.06	-0.22, 0.34	60	0.07	-0.20, 0.33	60	-0.11	-0.36, 0.16
Components									
Systolic blood pressure	54	**0.34**	**0.06, 0.57**	54	0.13	-0.16, 0.40	54	0.20	-0.10, 0.45
Diastolic blood pressure	54	0.26	-0.03, 0.551	54	0.10	-0.19, 0.37	54	0.18	-0.11, 0.44
HbA1c	53	**0.30**	**0.01, 0.53**	53	0.16	-0.14, 0.42	53	-0.04	-0.33, 0.25
Total cholesterol	55	0.15	-0.13, 0.42	55	0.06	-0.22, 0.34	55	-0.09	-0.36, 0.20
HDL cholesterol	55	0.22	-0.07, 0.47	55	-0.17	-0.43, 0.12	55	0.00	-0.28, 0.28
BMI	53	0.10	-0.20, 0.37	53	0.26	-0.03, 0.51	53	0.00	-0.29, 0.29
DHEA-S 30-39	33	0.04	-0.34, 0.42	33	-0.09	-0.46, 0.30	33	0.02	-0.36, 0.40
DHEA-S 40-50	22	0.03	-0.47, 0.52	22	-0.39	-0.73, 0.15	22	**-0.69**	**-0.88, -0.28**
C-reactive protein	55	-0.09	-0.36, 0.20	55	0.12	-0.17, 0.38	55	-0.09	-0.36, 0.20
Interleukin-6	55	0.02	-0.26, 0.30	55	-0.07	-0.34, 0.22	55	-0.01	-0.29, 0.27
Albumin	55	**0.46**	**0.20, 0.65**	55	0.08	-0.21, 0.35	55	-0.09	-0.36, 0.19

HDL: high-density lipoprotein; HbA1c: glycosylated hemoglobin; DHEA-S: dehydroepiandrosterone sulfate
bold: p-value <0.05
† adjusted for US birth, age, abstinence, time to analysis, exercise, job strain
†† adjusted for US birth, age, abstinence, time to analysis, exercise
††† adjusted for US birth, age, abstinence, time to analysis, income

Figure 3.1. Adjusted boxplots of mean semen parameters for each allostatic load level.
A. Sample high-risk cut points†



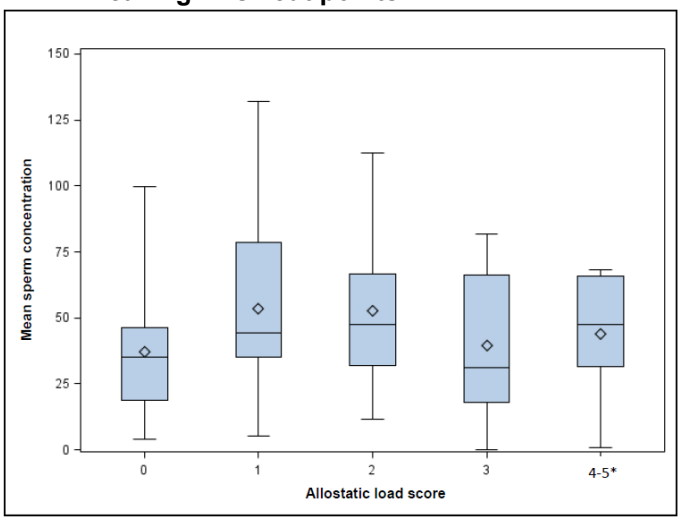


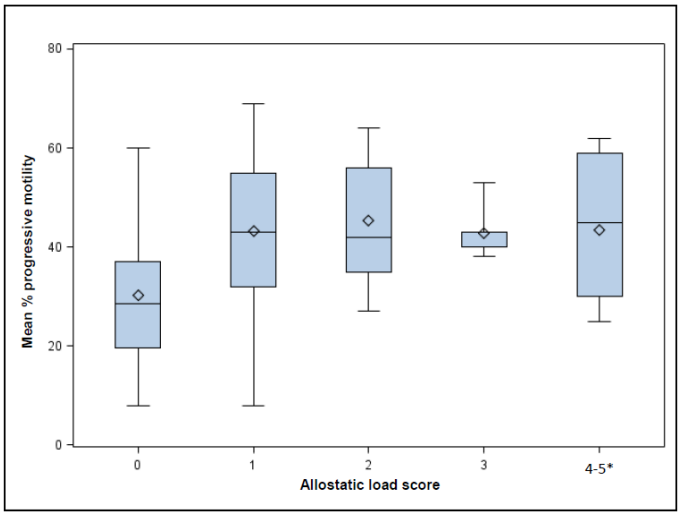


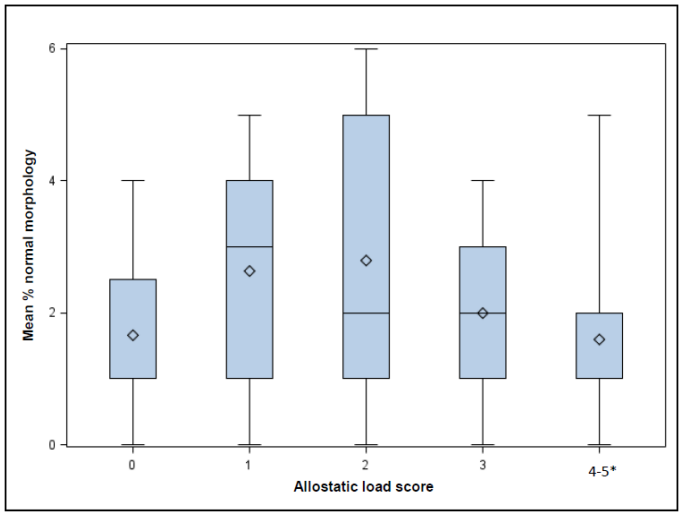
† Allostatic load (AL)=0, n=6; AL=1, n=10; AL=2, n=18; AL=3, n=12; AL=4, n=5; AL=5, n=5; AL=6-7, n=5

* combined because only 1 subject in level 7

B. Clinical high-risk cut points†







† Allostatic load (AL)=0, n=12; AL=1, n=19; AL=2, n=15; AL=3, n=5; AL=4-5, n=10
* combined because only 2 subjects in level 5

Supplemental Table 3.1. Summary of published articles on stress and sperm concentration, motility, and morphology.

First author, publication date	N	Population	Location	Stress exposure	Association with concentration	Association with % motility	Association with % normal morphology	Covariates
Abu-Musa, 2007	10,782	Andrology lab	Lebanon	Civil war	Negative	No association	Negative	None
Auger, 2001	1001	Fertile men	Denmark, Finland, France, Scotland	Level of stress in the last 3 months	N/A	N/A	Negative	None
Bhongade, 2015	70	IVF clinic	India	Hospital Anxiety Depression Score	N/A	Negative	Negative	Age, abstinence
Bigelow, 1998	845	Andrology lab	Canada	Question on perceived emotional stress related to job	No association	Negative	Negative	Age, abstinence, smoking, caffeine, season
Clarke, 1999	31	IVF clinic	USA	Spielberger State Anxiety Inventory (STAI), perceived stress over providing sample, perceived importance of providing a sample, general stress related to friends, family, work, home, finances	Negative for perceived importance of producing semen sample; no association for other stress variables	Negative for perceived importance of producing semen sample; no association for other stress variables	N/A	None
Drudy, 1994	125	IVF clinic	Ireland	IVF procedure	No association	Positive	N/A	None
Eskiocak, 2005	34	Medical students	Turkey	Exams	Negative	Negative	Negative	None
Fenster, 1997	157	General population	USA	Job Content Questionnaire (JCQ), stressful life events	No association	Negative for recent death of a close family member	No association	Age, abstinence, alcohol, smoking
Fukuda, 1996	27	IVF clinic	Japan	Earthquake	No association	Negative	N/A	None

Gerhard, 1992	225	IVF clinic	Germany	Stress questionnaires related to professional and personal lives	No association	No association	Negative	None
Giblin, 1988	28	Healthy men	USA	Life Stress Questionnaire, Life Events Inventory (LEI)	N/A	No association	Negative	None
Gollenberg, 2008	744	Fertile men	USA	Stressful life events	Negative	Negative	Negative	Age, abstinence, center, race, education, fever, time to analysis
Gurhan, 2009	80	IVF clinic	Turkey	STAI, Beck Depression Inventory	No association	No association	N/A	None
Hammond, 1990	198	IVF clinic	USA	IVF/IUI procedure	No association	No association	No association	None
Harrison, 1987	500	IVF clinic	Australia	IVF procedure	Negative	Negative	No association	None
Hjollund--job stress, 2004	399	First-time pregnancy planners	Denmark	JCQ	No association	No association	No association	Age, abstinence, center, alcohol, reproductive disorders, season, time of day
Hjollund--psych stress, 2004	418	First-time pregnancy planners	Denmark	General Health Questionnaire	No association	No association	No association	Age, abstinence, center, smoking, alcohol, caffeine, reproductive disorders, BMI, trade union, season, time of day

Hubert, 1985	101	IVF clinic	Germany	Fragebogen zur Abschätzung des Psychosomatischen Krankheitsgeschehens (stressful work conditions)	No association	No association	No association	None
Janevic, 2014	196	General population	USA	Perceived Stress Scale (PSS), LEI, JCQ	Negative for PSS; no association for LEI or job strain	Negative for PSS and LEI; no association for job strain	Negative for PSS and LEI; no association for job strain	Age, abstinence, race, education, income, smoking, BMI, STI, hazardous exposures
Jurewicz, 2014	327	IVF clinic	Poland	PSS, Subjective Work Characteristics Questionnaire (SWCQ), APGAR Family Scale	No association	Negative for APGAR; no association for PSS or SWCQ	No association	Age, abstinence, smoking, alcohol, BMI, past diseases, duration of infertility
Kentenich, 1992	180	IVF clinic	Germany	IVF procedure	Negative	No association	Negative	None
Lampiao, 2009	15	Medical students	Malawi	Exams	Negative	No association	N/A	None
Li, 2013	1346	General population	China	Psychological stress level question	No association	No association	Negative	Age, abstinence, region, BMI, season, time of day, fertility status
Lopez-Teijon, 2007	972	General population	Spain	STAI	Negative	No association	No association	None

Nordkap, 2016	1215	Military recruits from the general population	Denmark	4 perceived stress questions from the Copenhagen Psychosocial Questionnaire	Negative	No association	No association	Age, abstinence, smoking, alcohol, caffeine, physical fitness, STI, maternal smoking, maternal education, time to analysis
Nouri, 2014	78	IVF clinic	Austria	Fertility Problem Inventory	No association	No association	N/A	Age, fertility status
Poland, 1986	53	Medical students and general pop	USA	Exams	Positive	No association	N/A	None
Pook, 1999	69	Andrology lab	Germany	Infertility Distress Scale (IDS)	Negative	Negative	No association	Abstinence
Pook, 2004	120	Andrology lab	Germany	IDS	Negative	N/A	N/A	None
Ragni, 1992	84	IVF clinic	Italy	IVF procedure	Negative	Negative	N/A	None
Vellani, 2013	179	IVF clinic and general pop	Italy	STAI	Negative	Negative	N/A	None
Zorn, 2002	38	IVF clinic	Slovenia	Brief war	No association	Negative	No association	None
Zorn, 2008	1076	IVF clinic	Slovenia	Stressful life events, WHO Well-Being Index (depression), Zung's Anxiety Scale Inventory	Negative	No association	No association	Age, abstinence, smoking, cryptorchidism, varicocele

IVF: *in vitro* fertilization; IUI: intrauterine insemination

Supplemental Table 3.2. Biomarker analyses, Columbia University Medical Center Core Biomarkers Laboratory*.

	Specimen type	Method	Instrument	Manufacturer	Quantification limit	Inter-assay precision	Intra-assay precision
Glycosylated hemoglobin	whole blood	colorimetric/ turbidimetric assay	Cobas Integra 400 Plus	Roche Diagnostics (Indianapolis, IN)	not available	2.80%	1.50%
Total cholesterol	serum	colorimetric assay	Cobas Integra 400 Plus	Roche Diagnostics (Indianapolis, IN)	0.116 mg/dL	1.90%	0.51%
HDL cholesterol	serum	colorimetric assay	Cobas Integra 400 Plus	Roche Diagnostics (Indianapolis, IN)	3 mg/dL	1.00%	1.13%
DHEA-S	serum	chemiluminescence enzyme immunoassay	Immulite 1000	Siemens Healthcare Diagnostics (Deerfield, IL)	3 µg/dL	not available	5.00%
High-sensitivity C-reactive protein	serum	turbidimetric assay	Cobas Integra 400 Plus	Roche Diagnostics (Indianapolis, IN)	0.1 mg/dL	3.10%	1.30%
High-sensitivity Interleukin-6	serum	enzyme-linked immunosorbent assay	ELISA	R&D Systems (Minneapolis, MN)	0.039 pg/mL	8.10%	7.80%
Albumin	serum	colorimetric assay	Cobas Integra 400 Plus	Roche Diagnostics (Indianapolis, IN)	0.2 g/dL	2.30%	1.90%
Testosterone	serum	chemiluminescence enzyme immunoassay	Immulite 1000	Siemens Healthcare Diagnostics (Deerfield, IL)	15 ng/dL	not available	8.90%

HDL: high-density lipoprotein; DHEA-S: dehydroepiandrosterone sulfate
*All blood samples were run simultaneously to avert a batch effect.

Supplemental Table 3.3. Spearman correlations among allostatic load indicators.

Allostatic load indicator	1	2	3	4	5	6	7	8	9	10	11
1 Systolic blood pressure											
correlation	1										
p-value											
n	60										
2 Diastolic blood pressure											
correlation	***0.79***	1									
p-value	***<.0001***										
n	***60***	60									
3 Glycosylated hemoglobin											
correlation	-0.06	0.06	1								
p-value	0.67	0.66									
n	58	58	59								
4 Total cholesterol											
correlation	0.05	0.1	***0.33***	1							
p-value	0.68	0.44	***0.01***								
n	60	60	***59***	61							
5 High-density cholesterol											
correlation	**-0.23**	-0.11	**0.22**	0.12	1						
p-value	**0.08**	0.41	**0.09**	0.36							
n	**60**	60	**59**	61	61						
6 Body mass index											
correlation	***0.38***	**0.25**	0.11	0.05	***-0.46***	1					
p-value	***0.003***	**0.06**	0.42	0.73	***0.0003***						

n	***59***	**59**	57	59	***59***	59					
7 Dehydroepiandrosterone sulfate, age 30-39											
correlation	-0.11	-0.06	-0.18	0.12	0.02	-0.29	1				
p-value	0.53	0.72	0.32	0.5	0.9	0.09					
n	35	35	34	35	35	34	35				
8 Dehydroepiandrosterone sulfate, age 40-50											
correlation	0.003	-0.34	-0.17	-0.22	0.02	-0.02	.	1			
p-value	0.99	0.1	0.42	0.27	0.94	0.94	.				
n	25	25	25	26	26	25	0	26			
9 C-reactive protein											
correlation	0.1	0.14	0.12	-0.04	-0.21	***0.51***	-0.07	0.04	1		
p-value	0.43	0.28	0.37	0.79	0.11	***<.0001***	0.7	0.86			
n	60	60	59	61	61	***59***	35	26	61		
10 Interleukin-6											
correlation	-0.07	0.12	-0.06	0.05	-0.08	0.005	-0.03	***-0.48***	**0.25**	1	
p-value	0.6	0.37	0.66	0.72	0.54	0.97	0.85	***0.01***	**0.05**		
n	60	60	59	61	61	59	35	***26***	**61**	61	
11 Albumin											
correlation	0.17	0.14	0.13	***0.28***	0.21	-0.13	0.07	***0.44***	**-0.24**	-0.07	1
p-value	0.19	0.29	0.32	***0.03***	0.1	0.34	0.7	***0.03***	**0.07**	0.6	
n	60	60	59	***61***	61	59	35	***26***	**61**	61	61

bold italic: p-value <0.05
bold: p-value <0.10

Supplemental Table 3.4. Spearman correlations among semen parameters (n=61).

Semen outcome	1	2	3
1 Sperm concentration			
correlation	1		
p-value			
2 % Progressive motility			
correlation	0.33	1	
p-value	0.01		
3 % Normal morphology			
correlation	0.27	0.41	1
p-value	0.03	0.001	

Supplemental Table 3.5. Associations between allostatic load (AL) and measures of recent stress and depression.

	n	Spearman correlation coefficient	p-value
PSS	61	-0.04	0.77
CES-D	60	-0.18	0.17
		mean AL score (SD)	
LEI			0.16
no adverse events	45	2.80 (1.85)	
any adverse events	15	2.07 (1.16)	
Job strain			0.97
low	43	2.60 (1.79)	
high	12	2.58 (1.38)	

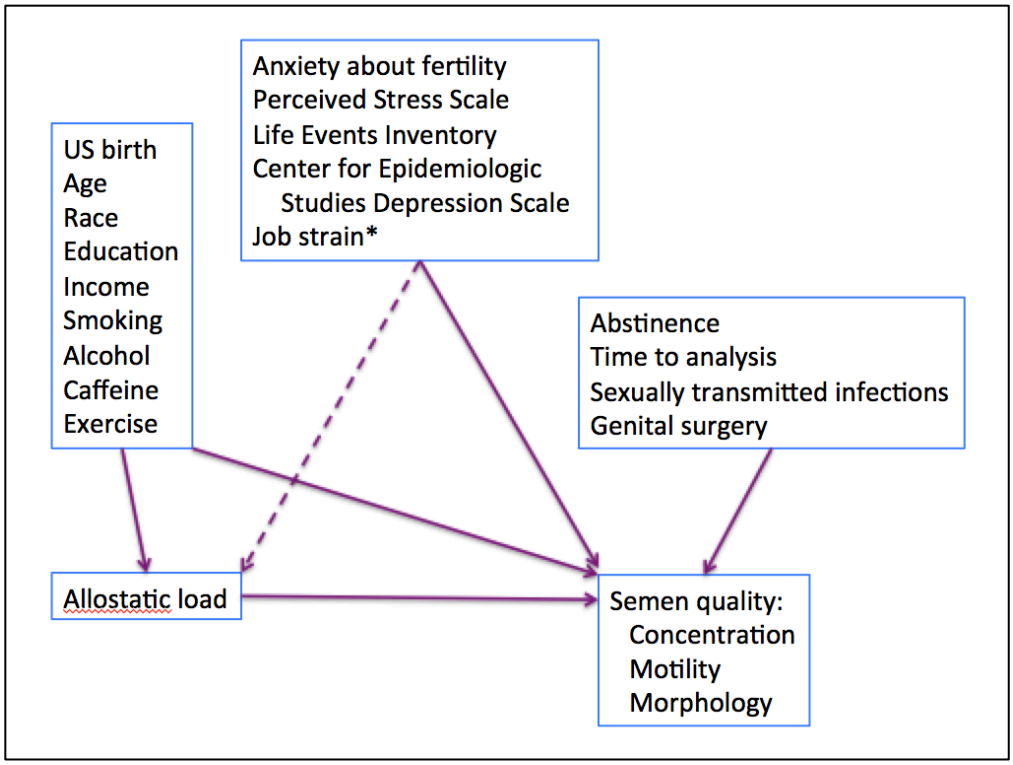
PSS: Perceived Stress Scale; CES-D: Center for Epidemiologic Studies Depression Scale; LEI: Life Events Inventory

Supplemental Table 3.6. Characteristics of subjects with AL=0 vs. others.

	AL=0 (n=6)	AL>0 (n=55)	
	mean (SD)	mean (SD)	p-value
Age	37.7 (5.6)	39.0 (6.0)	0.61
BMI (continuous)	25.8 (2.5)	28.5 (5.2)	0.22
Concentration (10^6/mL)	41.2 (29.7)	48.1 (29.2)	0.59
% Progressive motility	26.8 (12.0)	42.8 (13.8)	0.01
% Normal morphology	1.5 (1.4)	2.4 (1.7)	0.25
Testosterone (ng/dL)	523.3 (192.6)	391.8 (142.8)	0.04
FSH (mIU/mL)	4.0 (2.2)	4.6 (2.5)	0.56
	n (percent)	n (percent)	p-value
US birth			0.69
Yes	4 (66.7)	32 (58.2)	
No	2 (33.3)	23 (41.8)	
Race			0.75
White	4 (66.7)	33 (60.0)	
Black	0 (0)	4 (7.3)	
Hispanic	2 (33.3)	13 (23.6)	
Other	0 (0)	5 (9.1)	
Education			0.72
<Bachelors degree	2 (33.3)	15 (27.3)	
Bachelors degree	1 (16.7)	18 (32.7)	
>Bachelors degree	3 (50.0)	22 (40.0)	
Income			0.45
<$100,000	3 (50.0)	19 (35.2)	
$100-150,000	0 (0)	11 (20.4)	
>$150,000	3 (50.0)	24 (44.4)	
BMI			0.79
<25	2 (33.3)	15 (27.3)	
≥25 to <30	3 (50.0)	23 (41.8)	
≥30 to <35	1 (16.7)	9 (16.4)	
≥35	0 (0)	8 (14.6)	
Current smoking			0.49
Yes	0 (0)	4 (7.3)	
No	6 (100)	51 (92.7)	
Exercise			0.16
None	0 (0)	6 (11.1)	
>0 to <4 hours/week	3 (50.0)	14 (25.9)	
4 to 8 hours/week	0 (0)	20 (37.0)	
>8 hours/week	3 (50.0)	14 (25.9)	
Job strain			0.22
Low	5 (100)	38 (76.0)	
High	0 (0)	12 (24.0)	
Alcohol			0.12
None	2 (33.3)	6 (10.9)	
Any	4 (66.7)	49 (89.1)	

AL: allostatic load score; BMI: body mass index

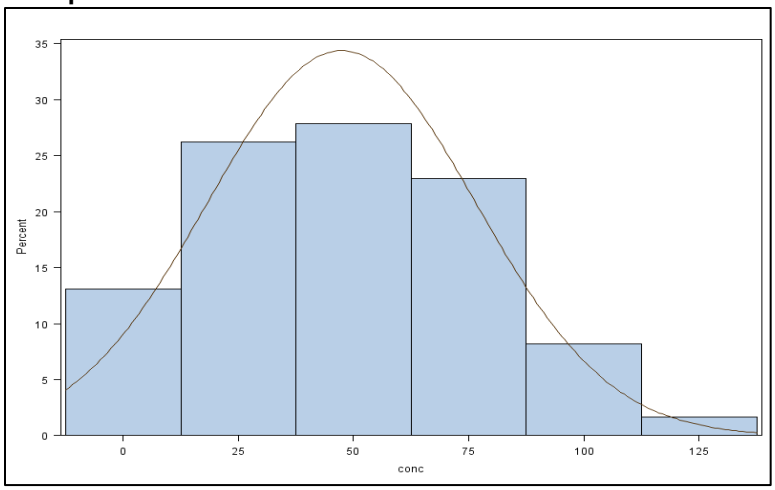
Supplemental Figure 3.1. Hypothesized causal diagram of the relationship between allostatic load, semen outcomes, and potential covariates.



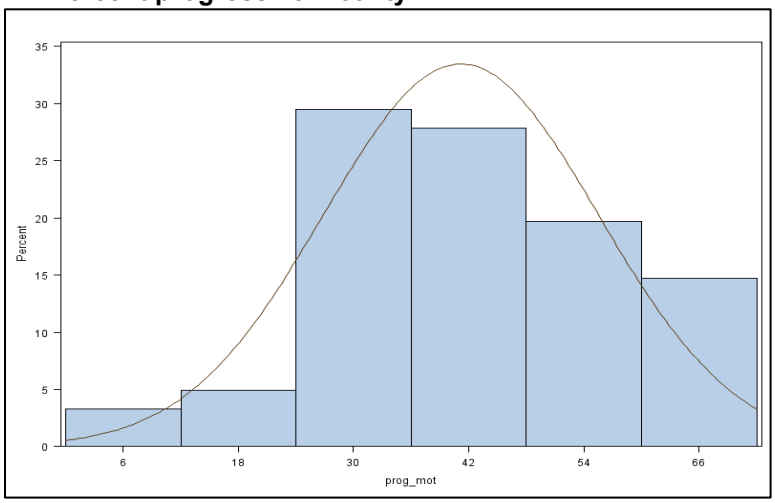
* Although allostatic load theoretically represents cumulative stress over the life course, some of its components may be influenced by current stress, hence the dotted line

Supplemental Figure 3.2. Histograms of semen outcomes measures.

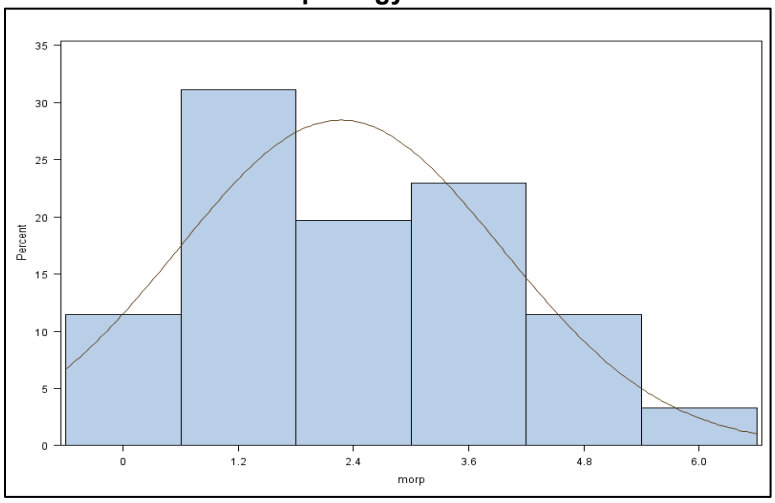
A. Sperm concentration



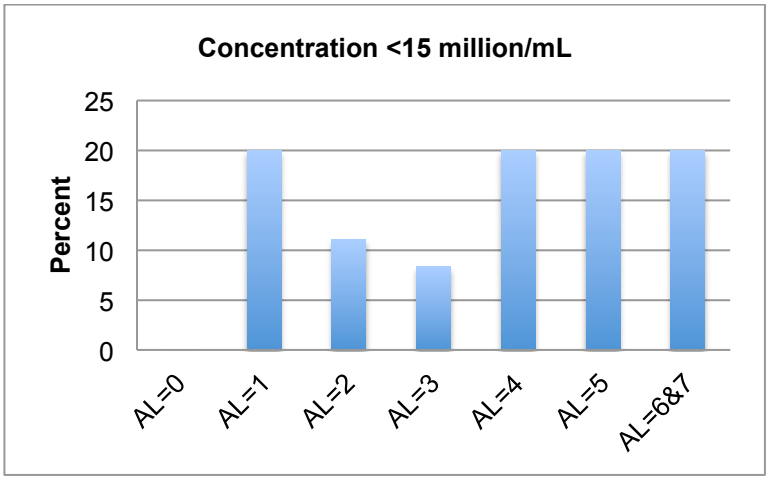
B. Percent progressive motility

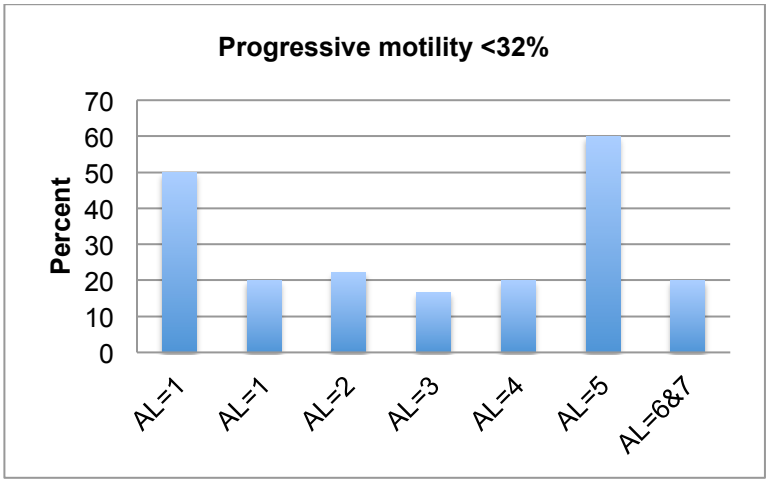


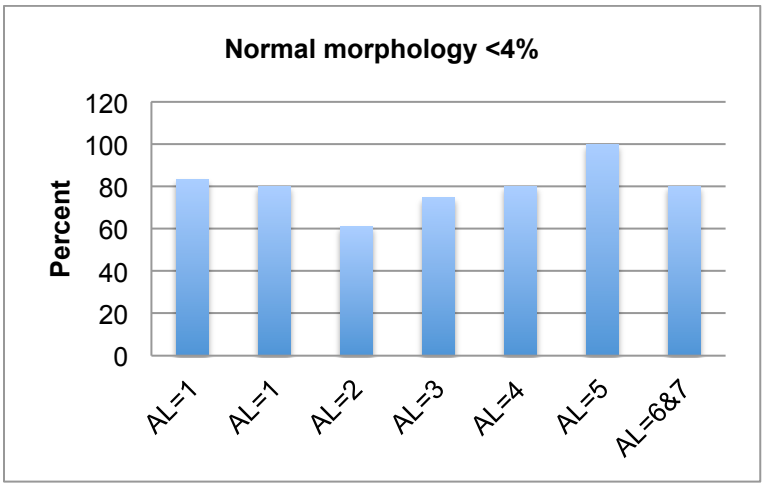
C. Percent normal morphology



Supplemental Figure 3.3. Percentages of subjects in each allostatic load category below World Health Organization reference levels for concentration, motility, and morphology.
A. Sample high-risk cut points†

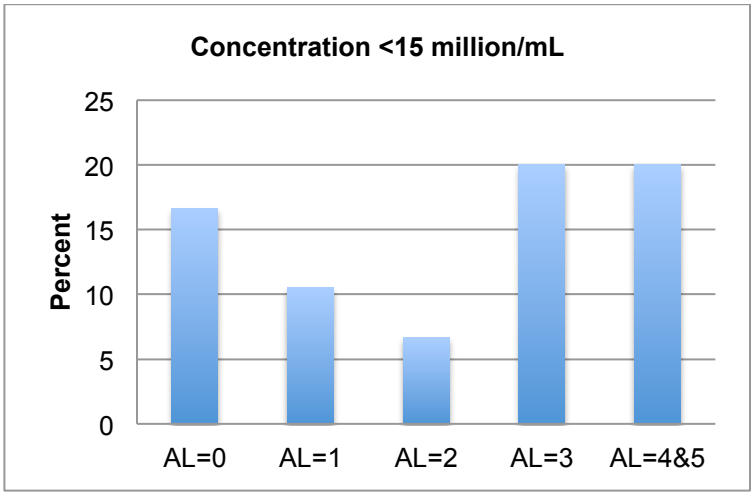


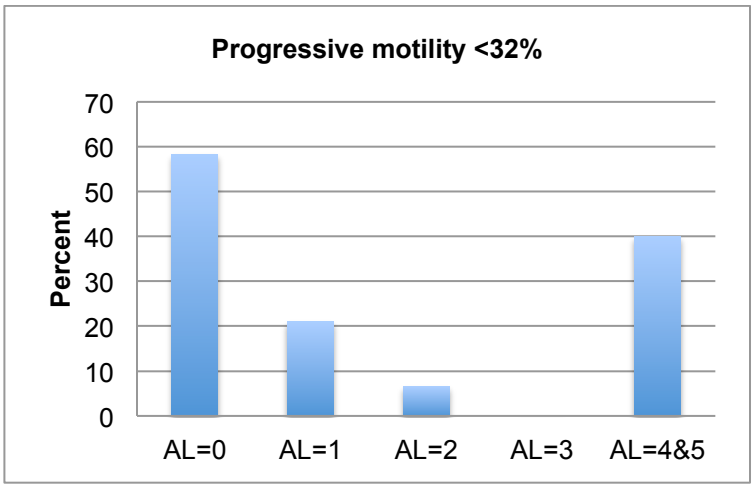


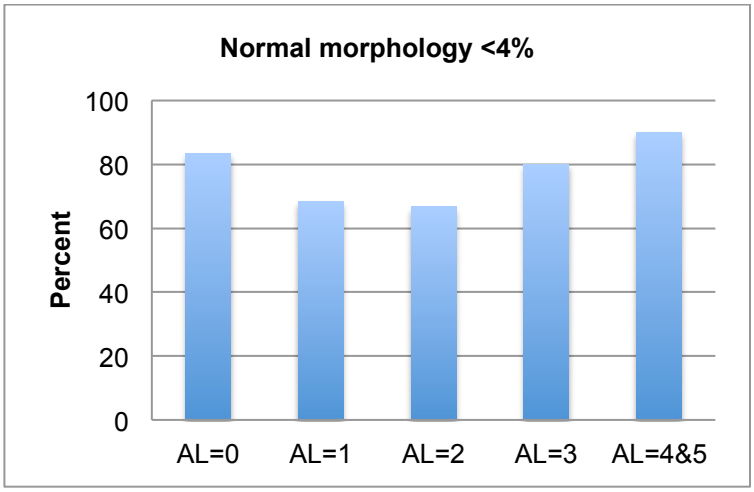


† Allostatic load (AL)=0, n=6; AL=1, n=10; AL=2, n=18; AL=3, n=12; AL=4, n=5; AL=5, n=5; AL=6&7 (combined because only 1 subject in level 7), n=5

B. Clinical high-risk cut points†

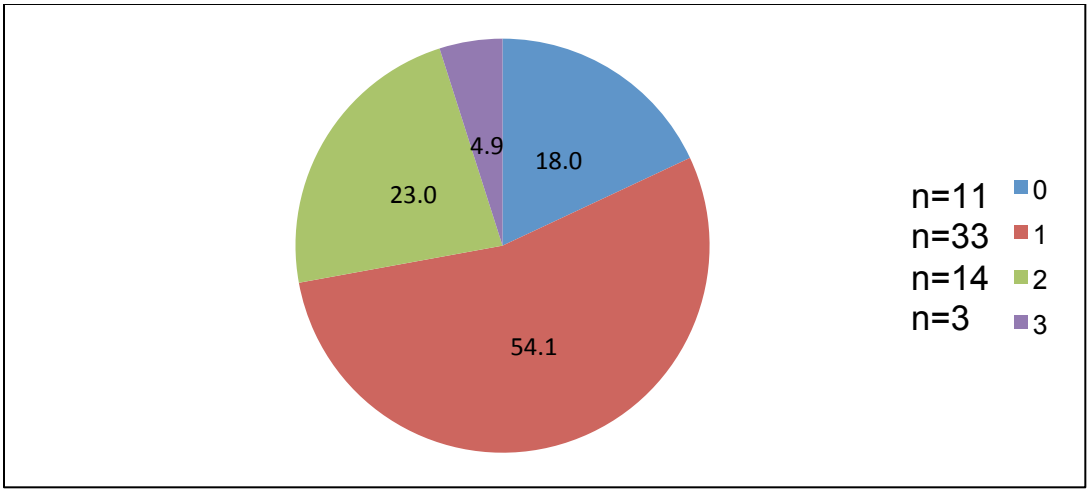






† Allostatic load (AL)=0, n=12; AL=1, n=19; AL=2, n=15; AL=3, n=5; AL=4&5 (combined because only 2 subjects in level 5), n=10

Supplemental Figure 3.4. Percentage of subjects meeting 0, 1, 2, or 3 World Health Organization subfertility criteria.



CONCLUSION

Because semen quality is not only a measure of male fertility but also a potential indicator of male health, it is important to understand predictors of poor semen quality and attempt to identify modifications in behavior and environment that may improve it. The results of my dissertation research impart as much about the relationship of adiposity and stress to semen quality as they do about considerations for future research in this area. Following are some caveats and recommendations.

Check for nonlinear associations.

The main finding of my systematic review was similar to that of Sermondade et al.'s meta-analysis(39). While they reported a J-shaped relationship in which under- and overweight/obese men were more likely to be azoospermatic or oligospermatic (to have no or low sperm count) compared to normal weight men, we concluded that the relationship between body mass index (BMI) and sperm concentration was likely to have an inverse U-shape. Most of the studies I examined found no association between BMI and sperm concentration, and the ones that found a negative association were more often conducted among populations with higher proportions of overweight and obese men. Studies that included only young men, which tended to have lower mean BMI, showed no associations and the single study that reported a positive association was conducted among a mixed-age group of men with a low mean BMI. It is therefore important not only to test for nonlinear relationships between BMI and semen outcomes, but to insure that there is adequate distribution of BMI within the sample population to detect nonlinearity at the high and low ends of the curve.

Cross-sectional studies may not tell the whole story.

Although it is convenient—especially in semen quality studies—to collect cross-sectional data or mine the records of fertility clinics and andrology labs for results that can be linked to subjects' demographic information, a true understanding of how risk factors affect sperm concentration, motility, and morphology—as well as other parameters that were not covered in

my studies, such as DNA fragmentation—requires a longitudinal design. While the division, proliferation, and maturation of sperm stem cells into spermatozoa occurs in less than 3 months, factors that affect the quantity and quality of those stem cells, including the number and functional health of the Sertoli and Leydig cells in the testes, may date back to the prenatal period.

A key example of what is missed in cross-sectional studies is my finding relating to BMI and sperm concentration in the Study of the Environment and Reproduction. When linked to data from Child Health and Development Studies, the combined longitudinal data set spanned more than 4 decades and included information from various developmental stages. If I had only analyzed the data cross-sectionally, I would have concluded that there was no association between adiposity and semen quality. Because I had access to birth weight and childhood adiposity measures, however, I was able to tell a different story that revealed potentially important information about the effects of the intrauterine environment and of early childhood overweight and obesity. Specifically, higher birth weight for gestational age is associated with increased sperm concentration, likely because more Sertoli cells, which give rise to sperm stem cells, are laid down in the testes of fetuses that have the opportunity to maximize their growth potential(161). Postnatally, however, the direction of the association changes. This suggests that early life adiposity may interfere with the development of testosterone-producing Leydig cells, which undergo crucial transformations in early childhood, and thereby impede sperm production in adulthood.

Similarly, had I only looked at the association between BMI at the time of semen collection and sperm motility, I would have concluded that there was no association. But having adiposity measures at two time points earlier in adulthood allowed me to see a negative association between obesity in subjects' 20s and motility at mean age 43 (among those overweight at 4 years, the relationship was already apparent). The association was attenuated at later ages, suggesting that it resulted from an accumulation of damage among those who had high BMI for the longest period of time, an interpretation borne out in the cumulative analysis.

Pay attention to puberty.

The years leading up to puberty and throughout adolescence are potentially critical periods in which adiposity or other exposures might negatively affect future semen quality because both Sertoli and Leydig cells undergo proliferation and maturation during this time(158, 162). Unfortunately, my data set lacked information adiposity measures between age 4 and subjects' 20s, so I was unable to investigate associations within this important developmental window. In addition to collecting data at regular intervals so as to avoid having such a frustrating gap, future longitudinal studies should collect information on sexual development, such as Tanner stages and date of first ejaculation. Having a marker of sexual maturity would permit an analysis to be structured according to biological rather than chronological age (e.g., time pre- or post-first ejaculation), which may be more relevant in the context of semen quality research(238).

Collect serial semen samples.

In addition to collecting prenatal data (including paternal semen samples), as well as anthropometric measures, biological samples, behavioral data, and assessments of physical and sexual development at regular intervals, the ideal longitudinal semen quality study would include serial semen samples collected from adolescence through adulthood. Having repeated measures of both exposures and outcomes would allow subjects to serve as their own controls and permit the study of changes in BMI or other exposures (e.g., exercise, diet, sleep) on semen quality. This method was used in some of the studies I reviewed that assessed the relationship between stress due to infertility treatment and semen quality(180, 187, 188, 193, 201). In the BMI literature, it has only been used to assess the effect of weight loss on semen quality among severely obese men through either a structured weight-loss program(77) or bariatric surgery(239-243). Large studies of this nature could provide valuable information about whether or not a change in a particular exposure is associated with a change in semen quality. Repeated semen quality measures could also provide an alternative means of assessing critical period effects. The method I used—adding sequential adiposity measures into the same model--was severely limited by the collinearity of the predictors. With repeated exposure and outcome measures, it would be

possible to assess whether or not there is a critical period in the life course when a change in exposure might have a lasting beneficial or deleterious effect on semen quality.

Think beyond the World Health Organization (WHO).

Studies of semen quality—especially those that rely on data collected at fertility clinics or andrology labs—generally restrict themselves to the conventional semen parameters for which the WHO has published lower reference limits and which are therefore usually assessed as part of a routine fertility work-up. In addition to concentration, motility and morphology, these include volume, total sperm count, and vitality(147). Recent studies have indicated that other characteristics, including sperm DNA fragmentation (reviewed in (244, 245)), sperm epigenetics (reviewed in (246, 247)), sperm telomere length(248-251), reactive oxygen species produced by sperm(252, 253), and presence of inflammatory cytokines in seminal fluid(254, 255) may also be relevant outcomes for semen quality research. In all cases, efforts should be made to measure biomarkers in the seminal fluid, as concentrations may differ between blood and semen(224, 226, 227).

Seek a biological explanation.

Studies that report negative associations between psychosocial stress or life event stress and semen quality have played an important role in confirming the observed phenomenon of reduced fertility in times of poverty, war, and famine(256-259). However they do not provide any information on which biological systems are compromised by stress and how those systems might be linked to the regulation of semen quality. Without this information, the only public health message to result from these studies is to “avoid stress.” If, as Last writes, the definition of epidemiology includes the application of the results of our research “to the control of health problems”(260), it is essential that we design studies that yield more specific, practical information.

I designed my pilot study of allostatic load, theoretically a construct that represents cumulative stress, and semen quality with the aim of discovering which biological mechanisms

were most responsible for observed associations of stress with sperm concentration, motility, and morphology. Because allostatic load comprises biomarkers and physical measures that represent various regulatory systems in the body, it is a useful tool for probing possible biological pathways. (Whether it indeed represents a distinct construct from recent stress is debatable; in my study, the allostatic load components that were most strongly correlated with the semen outcomes tended to be those that fluctuated in response to proximal events, such as blood pressure, glycosylated hemoglobin, and serum albumin.) Although my study had multiple drawbacks, the findings illustrate this point. The full allostatic load scale was positively associated with motility, but when the allostatic load scale was split into its metabolic and non-metabolic subdomains, it became apparent that the positive association was driven by the metabolic subdomain. Within that subdomain, BMI had the strongest positive signal. In the case of sperm concentration, I found no association with the full allostatic load scale, but a significant positive association with the metabolic subdomain and a nonsignificant negative association with the non-metabolic subdomain. In this case, BMI contributed relatively little to the positive association; systolic blood pressure and glycosylated hemoglobin predominated. Were these results from a larger study with fewer limitations, they might lead to further research into how these particular symptoms connect to the dysregulation of sperm production that could eventually result in clinical recommendations.

The results of my studies of adiposity and stress as potentially modifiable risk factors for semen quality have reinforced my belief in the importance of a biologically-based life course approach to epidemiologic research. We can only hope to unravel some of the complexities of the interactions between not only age, period, and cohort, but also environment, behavior, heredity, and—most importantly—human biology if we have data that covers the entire life span. In the case of reproductive heath, where the cells that give rise to gametes are present from the prenatal period, and those gametes can transmit epigenetic changes to offspring, intergenerational effects also come into play, necessitating the establishment of multigenerational cohorts. This dissertation project has both enlightened me to the challenges inherent in the field

of reproductive epidemiology and inspired me to try to meet some of those challenges in my future research.

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